CHAPTER 1

COMMON PHYSICAL TECHNIQUES USED IN PURIFICATION

GENERAL REMARKS

Purity is a matter of degree. Other than adventitious contaminants such as dust, paper fibres, wax, cork, etc., that may have been incorporated into the sample during manufacture, all commercially available chemical substances are in some measure impure. Any amounts of unreacted starting material, intermediates, by-products, isomers and related compounds may be present depending on the synthetic or isolation procedures used for preparing the substances. Inorganic reagents may deteriorate because of defective packaging (glued liners affected by sulphuric acid, zinc extracted from white rubber stoppers by ammonia), corrosion or prolonged storage. Organic molecules may undergo changes on storage. In extreme cases the container may be incorrectly labelled or, where compositions are given, they may be misleading or inaccurate for the proposed use. Where any doubt exists it is usual to check for impurities by appropriate spot tests, or by recourse to tables of physical or spectral properties such as the extensive infrared and NMR libraries published by the Aldrich Chemical Co. The important question, then, is not whether a substance is pure but whether a given sample is sufficiently pure for some intended purpose. That is, are the contaminants likely to interfere in the process or measurement that is to be studied. By suitable manipulation it is often possible to reduce levels of impurities to acceptable limits, but absolute purity is an ideal which, no matter how closely approached, can never be attained. A *negative* physical or chemical test indicates only that the amount of an impurity in a substance lies below a certain level; no test can demonstrate that a specified impurity is entirely absent.

When setting out to purify a laboratory chemical, it is desirable that the starting material is of the best grade commercially available. Particularly among organic solvents there is a range of qualities varying from *laboratory chemical* to *spectroscopic*, *chromatographic* and *electronic* grades. Many of these are suitable for use as received. With many of the commoner reagents it is possible to obtain from the current literature some indications of likely impurities, their probable concentrations and methods for detecting them. However, in many cases complete analyses are not given so that significant concentrations of unspecified impurities may be present. See for example *Reagent Chemicals* (American Chemical Society Specifications, 8th edn, 1992), the American Chemical Society for Testing Materials D56-36, D92-46, and national pharmacopoeias. Other useful sources include *Ashford's Dictionary of Industrial Chemicals*, R.D.Ashford, Wavelength Publications Ltd, 1995 and references on pp.44-47 and pp. 61-62. For purification of proteins, see for example R.K.Scopes, *Protein Purification,* Springer-Verlag, New York, 3rd edn, 1994, and for nucleic acids see for example T.A.Brown, *Essential Molecular Biology - A Practical Approach* (2 vols), Oxford University Press 1991.

Abbreviations

To save space the following abbreviations have been generally used in Chapters 3, 4 and 5: abs (absolute), anhyd (anhydrous), aq (aqueous), atm (atmospheric), crystd (crystallised), crystn (crystallisation), crysts (crystallises), dec (decomposes), dil (dilute), distd (distilled), distn (distillation), evap (evaporate), evapd (evaporated), evapn (evaporation), filtd (filtered), h (hour[s]), pet ether (petroleum ether, ligroin), ppte (precipitate), ppted (precipitated), pptn (precipitation), satd (saturated), soln (solution), TLC (thin layer chromatography), HPLC (high pressure liquid chromatography), vac (vacuum), vol (volume). Other abbreviations used occasionally are self evident in meaning.

The following journals are designated by their initials:

Annalen Chem.	*A*	*J.Chem.Soc.Farad.Trans.*	*JCSFT*
Analyt.Biochem	*AB*	*J.Heterocyclic Chem.*	*JHC*
Anal.Chem.	*AC*	*J.Chromatography*	*JC*
Ber.deut.Chem.Ges. or Chem.Ber.	*B*	*J.IndianChem.Soc.*	*JICS*
Biochem.J.	*BJ*	*J.Inorg.Nucl.Chem.*	*JINC*
Biochem.Biophys.Res.Commun.	*BBRC*	*J.Org.Chem.*	*JOC*
Brit.J.Pharmacol.	*BJP*	*J.Phys.Chem.*	*JPC*
Bull.Acad.Sci.USSR	*BASU*	*Monatsh Chemie*	*M*
Helv.Chim.Acta	*HCA*	*Pure Appl.Chem.*	*PAC*
Fed.Eur.Biochem.Soc.Letters	*FEBS LETT*	*Synthesis*	*S*
Ind.Eng.Chem.(Anal.Ed.)	*IECAE*	*Synth.Commun.*	*SC*
J.Am.Chem.Soc.	*JACS*	*Tetrahedron*	*TET*
J.Biol.Chem.	*JBC*	*Tetrahedron Letters*	*TET LETT*
J.Chem.Phys.	*JCP*	*Trans.Faraday Soc.*	*TFS*
J.Chem.Soc.	*JCS*	*Zhur.Org.Khimii*	*ZOK*
J.Chem.Soc.Chem.Commun.	*JCSCC*	*Z.Physik.Chem.*	*ZPC*
J.Chem.Soc.Dalton Trans.	*JCSDT*		

Abbreviations of periodicals not included in this list are written in such a way that the periodical can be readily identified, e.g. *Acta Chem Scand* for Acta Chemica Scandinavica.

Purity of Substances

Solvents and substances that are specified as *pure* for a particular purpose may, in fact, be quite impure for other uses. Absolute ethanol may contain traces of benzene, which makes it unsuitable for ultraviolet spectroscopy, or plasticizers which make it unsuitable for use in solvent extraction.

Irrespective of the grade of material to be purified, it is essential that some criteria exist for assessing the degree of purity of the final product. The more common of these include:

1. Examination of physical properties such as:
   (a) Melting point, freezing point, boiling point, and the freezing curve (i.e. the variation, with time, in the freezing point of a substance that is being slowly and continuously frozen).
   (b) Density.
   (c) Refractive index at a specified temperature and wave-length. The sodium D line at 589.26 nm (weighted mean of D_1 and D_2 lines) is the usual standard of wavelength but results from other wavelengths can often be interpolated from a plot of refractive index versus $1/(\text{wavelength})^2$.
   (d) Absorption spectra (ultraviolet, visible, infrared, and nuclear magnetic resonance).
   (e) Specific conductivity. (This can be used to detect, for example, water, salts, inorganic and organic acids and bases, in non-electrolytes).
   (f) Optical rotation, optical rotatory dispersion and circular dichroism.
   (g) Mass spectroscopy.

2. Empirical analysis, for C, H, N, ash, etc.

3. Chemical tests for particular types of impurities, e.g. for peroxides in aliphatic ethers (with acidified KI), or for water in solvents (quantitatively by the Karl Fischer method).

4. Physical tests for particular types of impurities:
   (a) Emission and atomic absorption spectroscopy for detecting and determining metal ions.
   (b) Chromatography, including paper, thin layer, liquid (high, medium and normal pressure) and vapour phase.
   (c) Electron spin resonance for detecting free radicals.
   (d) X-ray spectroscopy.

(e) Mass spectroscopy.
(f) Fluorimetry.

5. Electrochemical methods (see Chapter 5 for macromolecules).

6. Nuclear methods which include a variety of radioactive elements as in organic reagents, complexes or salts.

A substance is usually taken to be of an acceptable purity when the measured property is unchanged by further treatment (especially if it agrees with a recorded value). In general, at least two different methods, such as recrystallisation and distillation, should be used in order to ensure maximum purification. Crystallisation may be repeated (from the same solvent or better from different solvents) until the substance has a constant melting point or absorption spectrum, and until it distils repeatedly within a narrow, specified temperature range.

With liquids, the refractive index at a specified temperature and wavelength is a sensitive test of purity. Note however that this is sensitive to dissolved gasses such as O_2, N_2 or CO_2. Under favourable conditions, freezing curve studies are sensitive to impurity levels of as little as 0.001 moles per cent. Analogous fusion curve or heat capacity measurements can be up to ten times as sensitive as this. With these exceptions, most of the above methods are rather insensitive, especially if the impurities and the substances in which they occur are chemically similar. In some cases, even an impurity comprising many parts per million of a sample may escape detection.

The common methods of purification, discussed below, comprise distillation (including fractional distillation, distillation under reduced pressure, sublimation and steam distillation), crystallisation, extraction, chromatographic and other methods. In some cases, volatile and other impurities can be removed simply by heating. Impurities can also sometimes be eliminated by the formation of derivatives from which the purified material is regenerated.

Safety in the Chemical Laboratory

Although most of the manipulations involved in purifying laboratory chemicals are inherently safe, care is necessary if hazards are to be avoided in the chemical laboratory. In particular there are dangers inherent in the inhalation of vapours and absorption of liquids and low melting solids through the skin. To the toxicity of solvents must be added the risk of their flammability and the possibility of eye damage. Chemicals, particularly in admixture, may be explosive. Compounds may be carcinogenic or otherwise deleterious to health. Present day chemical catalogues specifically indicate the particular dangerous properties of the individual chemicals they list and these should be consulted whenever the use of commercially available chemicals is contemplated. Radioisotopic labelled compounds pose special problems of human exposure to them and of disposal of laboratory waste. Purchased chemicals are sometimes accompanied by detailed information regarding their toxicity, safety handling procedures and the necessary precautions to be taken. These should be read carefully.

The commonest hazards are:

(1) Explosions due to the presence of peroxides formed by aerial oxidation of ethers and tetrahydrofuran, decahydronaphthalene, acrylonitrile, styrene and related compounds.
(2) Compounds with low flash points (below room temperature). Examples are acetaldehyde, acetone, acetonitrile, benzene, carbon disulphide, cyclohexane, diethyl ether, ethyl acetate and *n*-hexane.
(3) Contact of oxidising agents ($KMnO_4$, $HClO_4$, chromic acid) with organic liquids.
(4) Toxic reactions with tissues.

For detailed discussion, see *Bretherick's Handbook of Reactive Chemical Hazards*, Butterworths, London, 1990, Sax's *Dangerous Properties of Industrial Materials*, 8th edn, van Nostrand Reinhold, NY 1992.

The laboratory should at least be well ventilated and safety glasses should be worn, particularly during distillation and manipulations carried out under reduced pressure or elevated temperatures. With this in mind we have endeavoured to warn users of this book whenever greater than usual care is needed in handling chemicals. As a general rule, however, **all chemicals which users are unfamiliar with should be treated with extreme care and assumed to be highly flammable and toxic.** The safety of others in a

laboratory should always be foremost in mind, with ample warning whenever a potentially hazardous operation is in progress. Also, unwanted solutions or solvents should never be disposed of *via* the laboratory sink. The operator should be aware of the usual means for disposal of chemicals in her/his laboratories and she/he should remove unwanted chemicals accordingly. **Never mix organic liquids for disposal in the same container, and always keep halogenated waste solvents for disposal separate from other liquids.**

Further aspects of safety are detailed on p.29.

Trace Impurities in Solvents

Some of the more obvious sources of contamination of solvents arise from storage in metal drums and plastic containers, and from contact with grease and screw caps. Many solvents contain water. Others have traces of acidic materials such as hydrochloric acid in chloroform. In both cases this leads to corrosion of the drum and contamination of the solvent by traces of metal ions, especially Fe^{3+}. Grease, for example on stopcocks of separating funnels and other apparatus, e.g. greased ground joints, is also likely to contaminate solvents during extractions and chemical manipulation.

A much more general source of contamination that has not received the consideration it merits comes from the use of plastics for tubing and containers. Plasticisers can readily be extracted by organic solvents from PVC and other plastics, so that most solvents, irrespective of their grade (including spectrograde and ultrapure) have been reported to contain 0.1 to 5ppm of plasticizer [de Zeeuw, Jonkman and van Mansvelt *AB* **67** 339 *1975*]. Where large quantities of solvent are used for extraction (particularly of small amounts of compounds), followed by evaporation, this can introduce significant amounts of impurity, even exceeding the weight of the genuine extract and giving rise to spurious peaks in gas chromatography (for example of fatty acid methyl esters, Pascaud, *AB* **18** 570 *1967*). Likely contaminants are di(2-ethylhexyl)phthalate and dibutyl phthalate, but upwards of 20 different phthalic esters are listed as plasticisers as well as adipates, azelates, phosphates, epoxides, polyesters, trimellitates, and various heterocyclic compounds. These plasticisers would enter the solvent during passage through plastic tubing or from storage in containers or from plastic coatings used in cap liners for bottles. Such contamination could arise at any point in the manufacture or distribution of a solvent. The trouble with cap liners is avoidable by using corks wrapped in aluminium foil, although even in this case care should be taken because aluminium foil can dissolve in some liquids e.g. benzylamine and propionic acid.

Solutions in contact with polyvinyl chloride can become contaminated with trace amounts of lead, titanium, tin, zinc, iron, magnesium or cadmium from additives used in the manufacture and moulding of PVC.

N-Phenyl-2-naphthylamine is a contaminant of solvents and biological materials that have been in contact with black rubber or neoprene (in which it is used as an antioxidant). Although it was only an artefact of the separation procedure it has been isolated as an apparent component of vitamin K preparations, extracts of plant lipids, algae, livers, butter, eye tissue and kidney tissue [Brown *Chemistry in Britain* **3** 524 *1967*].

Most of the above impurities can be removed by prior distillation of the solvent, but care should be taken to avoid plastic or black rubber as much as possible.

Cleaning Apparatus

Laboratory glassware and Teflon equipment can be cleaned satisfactorily for most purposes by treating initially with a solution of sodium dichromate in concentrated sulphuric acid, draining, and rinsing copiously with distilled water. Where traces of chromium (adsorbed on the glass) must be avoided, a 1:1 mixture of concentrated sulphuric and nitric acid is a useful alternative. (*Used in a fumehood to remove vapour and with adequate face protection.*) Acid washing is also suitable for polyethylene ware but prolonged contact (some weeks) leads to severe deterioration of the plastic. For much glassware, washing with hot detergent solution, using tap water, followed by rinsing with distilled water and acetone, and heating to 200-300° overnight, is adequate. (Volumetric apparatus should not be heated: after washing it is rinsed with acetone, then hexane, and air-dried. Prior to use, equipment can be rinsed with acetone, then with petroleum ether or hexane, to remove the last traces of contaminants.) Teflon equipment should be soaked, first in acetone, then in petroleum ether or hexane for ten minutes prior to use.

For trace metal analyses, prolonged soaking of equipment in 1M nitric acid may be needed to remove adsorbed metal ions.

Soxhlet thimbles and filter papers may contain traces of lipid-like materials. For manipulations with highly pure materials, as in trace-pesticide analysis, thimbles and filter papers should be thoroughly extracted with hexane before use.
Trace impurities in silica gel for TLC can be removed by heating at 300° for 16h or by Soxhlet extraction for 3h with redistilled chloroform, followed by 4h extraction with redistilled hexane.

Sililation of Glassware and Plasticware
Sililation of apparatus makes it repellant to water and hydrophilic materials. It minimises loss of solute by adsorption onto the walls of the container. The glassware is placed in a desiccator containing dichloromethyl silane (1ml) in a small beaker and evacuated from 5min. The vacuum is turned off and air is introduced into the desiccator which allows the sililating agent to coat the glassware uniformly. The desiccator is then evacuated, closed and set aside for 2h. The glassware is removed from the desiccator and baked at 180° for 2h before use.
Plasticware is treated similarly except that it is rinsed well with water before use instead of baking. Note that dichloromethyl silane is highly **TOXIC** and **VOLATILE**, and the whole operation should be carried out in an efficient fumecupboard.
An alternative procedure used for large apparatus is to rinse it with a 5% solution of dichloromethyl silane in chloroform, then rinse several times with water before baking at 180°/2h (for glass) or drying in air (for plasticware). REPEL-SILANE (a solution of 2% w/v of dichloromethyl silane in 1,1,1-trichloroethane) is available commercially (LKB, Sweden).

DISTILLATION

One of the most widely applicable and most commonly used methods of purification of liquids or low melting solids (especially of organic chemicals) is fractional distillation at atmospheric, or some lower, pressure. Almost without exception, this method can be assumed to be suitable for all organic liquids and most of the low-melting organic solids. For this reason it has been possible in Chapter 3 to omit many procedures for purification of organic chemicals when only a simple fractional distillation is involved - the suitability of such a procedure is implied from the boiling point.
The boiling point of a liquid varies with the atmospheric pressure to which it is exposed. A liquid boils when its vapour pressure is the same as the external pressure on its surface, its normal boiling point being the temperature at which its vapour pressure is equal to that of a standard atmosphere (760mm Hg). Lowering the external pressure lowers the boiling point. For most substances, boiling point and vapour pressure are related by an equation of the form,

$$\log p = A + B/(t + 273),$$

where p is the pressure, t is in °C, and A and B are constants. Hence, if the boiling points at two different pressures are known the boiling point at another pressure can be calculated from a simple plot of log p *versus* $1/(t + 273)$. For organic molecules that are not strongly associated, this equation can be written in the form,

$$\log p = 8.586 - 5.703\ (T + 273)/(t + 273)$$

where T is the boiling point in °C at 760mm Hg. Table 1 gives computed boiling points over a range of pressures. Some examples illustrate its application. Ethyl acetoacetate, **b** 180° (with decomposition) at 760mm Hg has a predicted **b** of 79° at 8mm; the experimental value is 78°. Similarly 2,4-diaminotoluene, **b** 292° at 760mm, has a predicted **b** of 147° at 8mm; the experimental value is 148-150°. For self-associated molecules the predicted **b** are lower than the experimental values. Thus, glycerol, **b** 290° at 760mm, has a predicted **b** of 168° at 8mm: the experimental value is 182°.
For pressures near 760mm, the change in boiling point is given approximately by [Crafts *B* **20** 709 *1887*],

$$\hat{I}t = a(760 - p)(t + 273)$$

where $a = 0.00012$ for most substances, but $a = 0.00010$ for water, alcohols, carboxylic acids and other associated liquids, and $a = 0.00014$ for very low-boiling substances such as nitrogen or ammonia.

When all the impurities are non-volatile, simple distillation is an adequate purification. The observed boiling point remains almost constant and approximately equal to that of the pure material. Usually, however, some of the impurities are appreciably volatile, so that the boiling point progressively rises during the distillation because of the progressive enrichment of the higher-boiling components in the distillation flask. In such cases, separation is effected by fractional distillation using an efficient column.

The principle involved in fractional distillation can be seen by considering a system which approximately obeys *Raoult's law*. (This law states that the vapour pressure of a solution at any given temperature is the sum of the vapour pressures of each substance multiplied by its mole fraction in the solution.) If two substances, A and B, having vapour pressures of 600mm Hg and 360mm Hg, respectively, were mixed in a mole ratio of 2:1, the mixture would have (ideally) a vapour pressure of 520mm Hg and the vapour phase would contain 77% of A and 23% of B. If this phase was now condensed, the new liquid phase would, therefore, be richer in the volatile component A. Similarly, the vapour in equilibrium with this phase is still further enriched in A. Each such liquid-vapour equilibrium constitutes a "theoretical plate". The efficiency of a fractionating column is commonly expressed as the number of such plates to which it corresponds in operation. Alternatively, this information may be given in the form of the height equivalent to a theoretical plate, or HETP.

In most cases, systems deviate to a greater or less extent from Raoult's law, and vapour pressures may be greater or less than those calculated from it. In extreme cases, vapour pressure-composition curves pass through maxima or minima, so that attempts at fractional distillation lead finally to the separation of a constant-boiling (azeotropic) mixture and one (but not both) of the pure species if either of the latter is present in excess.

Techniques

Distillation apparatus consists basically of a distillation flask, usually fitted with a vertical fractionating column (which may be empty or packed with suitable materials such as glass helices or stainless-steel wool) to which is attached a condenser leading to a receiving flask. The bulb of a thermometer projects into the vapour phase just below the region where the condenser joins the column. The distilling flask is heated so that its contents are steadily vaporised by boiling. The vapour passes up into the column where, initially, it condenses and runs back into the flask. The resulting heat transfer gradually warms the column so that there is a progressive movement of the vapour phase-liquid boundary up the column, with increasing enrichment of the more volatile component. Because of this fractionation, the vapour finally passing into the condenser (where it condenses and flows into the receiver) is commonly that of the lowest-boiling components in the system. The conditions apply until all of the low-boiling material has been distilled, whereupon distillation ceases until the column temperature is high enough to permit the next component to distil. This usually results in a temporary fall in the temperature indicated by the thermometer.

The efficiency of a distillation apparatus used for purification of liquids depends on the difference in boiling points of the pure material and its impurities. For example, if two components of an ideal mixture have vapour pressures in the ratio 2:1, it would be necessary to have a still with an efficiency of at least seven plates (giving an enrichment of $2^7 = 128$) if the concentration of the higher-boiling component in the distillate was to be reduced to less than 1% of its initial value. For a vapour pressure ratio of 5:1, three plates would achieve as much separation.

In a fractional distillation, it is usual to reject the initial and final fractions, which are likely to be richer in the lower-boiling and higher-boiling impurities. The centre fraction can be further purified by repeated fractional distillation.

To achieve maximum separation by fractional distillation:

1. The column must be flooded initially to wet the packing. For this reason it is customary to operate a still at reflux for some time before beginning the distillation.

2. The reflux ratio should be high (i.e the ratio of drops of liquid which return to the distilling flask and the drops which distil over), so that the distillation proceeds slowly and with minimum disturbance of the equilibria in the column.

3. The hold-up of the column should not exceed one-tenth of the volume of any one component to be separated.

4. Heat loss from the column should be prevented but, if the column is heated to offset this, its temperature must not exceed that of the distillate in the column.

5. Heat input to the still-pot should remain constant.

6. For distillation under reduced pressure there must be careful control of the pressure to avoid flooding or cessation of reflux.

Distillation at Atmospheric Pressure

The distilling flask. To minimise superheating of the liquid (due to the absence of minute air bubbles or other suitable nuclei for forming bubbles of vapour), and to prevent bumping, one or more of the following precautions should be taken:

(a) The flask is heated uniformly over a large part of its surface, either by using an electrical heating mantle or, much better, by partial immersion in a bath somewhat above the boiling point of the liquid to be distilled.

(b) Before heating begins, small pieces of unglazed fireclay or porcelain (porous pot, boiling chips), pumice, carborundum, Teflon, diatomaceous earth, or platinum wire are added to the flask. These act as sources of air bubbles.

(c) The flask may contain glass siphons or boiling tubes. The former are inverted J-shaped tubes, the end of the shorter arm being just above the surface of the liquid. The latter comprise long capillary tubes sealed above the lower end.

(d) A steady slow stream of inert gas(e.g. N_2, Ar or He) is passed through the liquid.

(e) In some cases zinc dust can also be used. It reacts chemically with acidic or strongly alkaline solutions to liberate fine bubbles of hydrogen.

(f) The liquid in the flask is stirred mechanically. This is especially necessary when suspended insoluble material is present.

For simple distillations a Claisen flask (see, for example, Quickfit and Quartz Ltd cataloque of interchangeable laboratory glassware, Kontes Glass Co, Vineland, New Jersey, cat.no TG-15, Normschiff, Wertheim, Germany, Embell Scientific, Murwillumbah, NSW 2484, Australia) is often used. This flask is, essentially, a round-bottomed flask to the neck of which is joined another neck carrying a side arm. This second neck is sometimes extended so as to form a Vigreux column.
For heating baths, see Table 2 (p 33). For distillation apparatus on a semi-micro scale see Quickfit, Kontes and other glassware catalogues (above).

Types of columns and packings. A slow distillation rate is necessary to ensure that equilibrium conditions operate and also that the vapour does not become superheated so that the temperature rises above the boiling point. Efficiency is improved if the column is heat insulated (either by vacuum jacketing or by lagging) and, if necessary, heated to just below the boiling point of the most volatile component (an electrical heating tape is convenient for this purpose.) Efficiency of separation also improves with increase in the heat of vaporisation of the liquids concerned (because fractionation depends on heat equilibration at multiple liquid-gas boundaries). Water and alcohols are more easily purified by distillation for this reason.
Columns used in distillation vary in their shapes and types of packing. Packed columns are intended to give efficient separation by maintaining a large surface of contact between liquid and vapour. Efficiency of separation is further increased by operation under conditions approaching total reflux, i.e. under a high reflux ratio. Better control of reflux ratio is achieved by fitting a total condensation, variable take-off still-head (see, for example, catalogues by Quickfit and Quartz, or Kontes) to the top of the fractionating column. However, great care must be taken to avoid flooding of the column during distillation. The minimum number of theoretical plates for satisfactory separation of two liquids differing in boiling point by $\hat{I}t$ is approximately $(273 + t)/3\hat{I}t$, where t is the average boiling point in °C.

Some of the commonly used columns are:

Bruun column. A type of all-glass bubble-cap column.

Bubble-cap column. A type of plate column in which inverted cups (bubble caps) deflect ascending vapour through reflux liquid lying on each plate. Excess liquid from any plate overflows to the plate lying below it and ultimately returns to the flask. (For further details, see Bruun and Faulconer *Ind Eng Chem (Anal Ed)* **9** 247 *1937*). Like most plate columns, it has a high through-put, but a relatively low number of theoretical plates for a given height.

Dufton column. A plain tube, into which fits closely (preferably ground to fit) a solid glass spiral wound round a central rod. It tends to choke at temperatures above 100° unless it is lagged (Dufton *J Soc Chem Ind* (London) **38** 45T *1919*).

Hempel column. A plain tube (fitted near the top with a side arm) which is almost filled with a suitable packing, which may be of rings or helices.

Oldershaw column. An all-glass perforated-plate column. The plates are sealed into a tube, each plate being equipped with a baffle to direct the flow of reflux liquid, and a raised outlet which maintains a definite liquid level on the plate and also serves as a drain on to the next lower plate [see Oldershaw *Ind Eng Chem (Anal Ed)* **11** 265 *1941*].

Podbielniak column. A plain tube containing "Heli-Grid" Nichrome or Inconel wire packing. This packing provides a number of passage-ways for the reflux liquid, while the capillary spaces ensure very even spreading of the liquid, so that there is a very large area of contact between liquid and vapour while, at the same time, channelling and flooding are minimised. A column 1m high has been stated to have an efficiency of 200-400 theoretical plates (for further details, see Podbielniak *Ind Eng Chem (Anal Ed)* **13** 639 *1941*; Mitchell and O'Gorman *AC* **20** 315 *1948*).

Stedman column. A plain tube containing a series of wire-gauze discs stamped into flat, truncated cones and welded together, alternatively base-to-base and edge-to-edge, with a flat disc across each base. Each cone has a hole, alternately arranged, near its base, vapour and liquid being brought into intimate contact on the gauze surfaces (Stedman *Canad J Research B* **15** 383 *1937*).

Todd column. A column (which may be a Dufton type, fitted with a Monel metal rod and spiral, or a Hempel type, fitted with glass helices) which is surrounded by an open heating jacket so that the temperature can be adjusted to be close to the distillation temperature (Todd *Ind Eng Chem (Anal Ed)* **17** 175 *1945*).

Vigreux column. A glass tube in which have been made a number of pairs of indentations which almost touch each other and which slope slightly downwards. The pairs of indentations are arranged to form a spiral of glass inside the tube.

Widmer column. A Dufton column, modified by enclosing within two concentric tubes the portion containing the glass spiral. Vapour passes up the outer tube and down the inner tube before entering the centre portion. In this way flooding of the column, especially at high temperatures, is greatly reduced (Widmer *HCA* **7** 59 *1924*).

The packing of a column greatly increases the surface of liquid films in contact with the vapour phase, thereby increasing the efficiency of the column, but reducing its capacity (the quantities of vapour and liquid able to flow in opposite directions in a column without causing flooding). Material for packing should be of uniform size, symmetrical shape, and have a unit diameter less than one eighth that of the column. (Rectification efficiency increases sharply as the size of the packing is reduced but so, also, does the hold-up in the column.) It should also be capable of uniform, reproducible packing.

The usual packings are:

(a) **Rings.** These may be hollow glass or porcelain (Raschig rings), of stainless steel gauze (Dixon rings), or hollow rings with a central partition (Lessing rings) which may be of porcelain, aluminium, copper or nickel.

(b) **Helices.** These may be of metal or glass (Fenske rings), the latter being used where resistance to chemical attack is important (e.g. in distilling acids, organic halides, some sulphur compounds, and phenols). Metal single-turn helices are available in aluminium, nickel or stainless steel. Glass helices are less efficient, because they cannot be tamped to ensure uniform packing.

(c) **Balls.** These are usually glass.

(d) **Wire packing.** For use of "Heli-Grid" and "Heli-Pak" packings see references given for Podbielniak column. For Stedman packing, see entry under Stedman column.

Condensers. Some of the more commonly used condensers are:

Air condenser. A glass tube such as the inner part of a Liebig condenser. Used for liquids with boiling points above 90°. Can be of any length.

Allihn condenser. The inner tube of a Liebig condenser is modified by having a series of bulbs to increase the condensing surface. Further modifications of the bubble shapes give the Julian and Allihn-Kronbitter condensers.

Bailey-Walker condenser. A type of all-metal condenser fitting into the neck of extraction apparatus and being supported by the rim. Used for high-boiling liquids.

Coil condenser. An open tube, into which is sealed a glass coil or spiral through which water circulates. The tube is sometimes also surrounded by an outer cooling jacket.

Double surface condenser. A tube in which the vapour is condensed between an outer and inner water-cooled jacket after impinging on the latter. Very useful for liquids boiling below 40°.

Friedrichs condenser. A "cold-finger" type of condenser sealed into a glass jacket open at the bottom and near the top. The cold finger is formed into glass screw threads.

Graham condenser. A type of coil condenser.

Hopkins condenser. A cold-finger type of condenser resembling that of Friedrichs.

Liebig condenser. An inner glass tube surrounded by a glass jacket through which water is circulated.

Othmer condenser. A large-capacity condenser which has two coils of relatively large bore glass tubing inside it, through which the water flows. The two coils join at their top and bottom.

West condenser. A Liebig condenser with a light-walled inner tube and a heavy-walled outer tube, with only a narrow space between them.

Wiley condenser. A condenser resembling the Bailey-Walker type.

VACUUM DISTILLATION

This expression is commonly used to denote a distillation under reduced pressure lower than that of the normal atmosphere. Because the boiling point of a substance depends on the pressure, it is often possible by sufficiently lowering the pressure to distil materials at a temperature low enough to avoid partial or complete decomposition, even if they are unstable when boiled at atmospheric pressure.

Sensitive or high-boiling liquids should invariably be distilled or fractionally distilled under reduced pressure. The apparatus is essentially as described for distillation except that ground joints connecting the different parts of the apparatus should be greased with the appropriate vacuum grease. For low, moderately high, and very high temperatures Apiezon L, M and T, respectively, are very satisfactory. Alternatively, it is often preferable to avoid grease and to use thin Teflon sleeves in the joints. The distilling flask, must be supplied with a capillary bleed (which allows a fine stream of air, nitrogen or argon into the flask), and the receiver should be of the fraction collector type (e.g. a Perkin triangle, see Quickfit and Quartz Ltd interchangeable glassware catalogue, or Kontes Glass Co, Vineland, New Jersey, cat. no. TG-15). When distilling under vacuum it is very important to place a loose packing of glass wool above the liquid to buffer sudden boiling of the liquid. The flask should be not more than two-thirds full of liquid. The vacuum must have attained a steady state before the heat source is applied, and the temperature of the heat source must be raised *very slowly* until boiling is achieved.

If the pump is a filter pump off a high-pressure water supply, its performance will be limited by the temperature of the water because the vapour pressure of water at 10°, 15°, 20° and 25° is 9.2, 12.8, 17.5 and 23.8mm Hg respectively. The pressure can be measured with an ordinary manometer. For vacuums in the range 10^{-2}mm Hg (10μ) to 10mm Hg, rotary mechanical pumps (oil pumps) are used and the pressure can be measured with a Vacustat McLeod type gauge. If still higher vacuums are required, for example for high vacuum sublimations, a mercury diffusion pump is suitable. In principle, this pump resembles an ordinary water pump. It has a single, double or triple jet through which the mercury vapour and condensate pass. Such a pump can provide a vacuum up to 10^{-6} mm Hg. Two pumps can be used in series. For better efficiency these pumps are backed by a mechanical pump. The pressure is measured with a Pirani gauge. Where there is fear of contamination with mercury vapour, the mercury in the pumps can be replaced with vacuum oils, e.g. Apiezon type G or Silicone fluid (Dow Corning no. 702 or 703), which produce a vacuum range of 10^{-4} to 10^{-7}mm Hg depending on pump design and system used. These fluids are resistant to oxidation, are non-corrosive and are non-toxic. The gauge should be as close to the distillation apparatus as possible in order to obtain the distillation pressure as accurately as possible, thus minimising the pressure drop between the gauge and the apparatus.

In all cases, the pump is connected to the still through several traps to remove vapours. These traps may operate by chemical action, for example the use of sodium hydroxide pellets to react with acids, or by condensation, in which case empty tubes cooled in solid carbon dioxide-ethanol or liquid nitrogen (contained in wide-mouthed Dewar flasks) are used.

Special oil or mercury traps are available commercially and a liquid-nitrogen trap is the most satisfactory one to use between these and the apparatus. It has an advantage over liquid air or oxygen in that it is non-explosive if it becomes contaminated with organic matter. Air should not be sucked through the apparatus before starting a distillation or sublimation because this will cause liquid air to condense in the liquid nitrogen trap and a good vacuum cannot be readily achieved. Hence, it is advisable to degas the system for a short period before the trap is immersed into the liquid nitrogen (which is kept in a Dewar flask).

Kügelrohr Distillation. This is more like reverse molecular distillation. The apparatus (Büchi Glasapparat Fabrik, FLAWL, Switzerland) is made up of small glass bulbs (*ca* 4-5cm diameter) which are joined together *via* Quickfit joints at each pole of the bulbs. The liquid (or low melting solid) to be purified is placed in the first bulb of a series of bulbs joined end to end, and the system can be evacuated. The first bulb is heated in a movable furnace at a high temperature whereby most of the material distils into the second bulb (which is outside of the furnace). The furnace is then moved to the second bulb and the furnace temperature is reduced by *ca* 5° whereby the liquid in the second bulb distils into the third bulb (at this stage the first bulb is now out of the back of the furnace and the third and subsequent bulbs are outside the front of the furnace). The furnace temperature is lowered by a further *ca* 5° and moved to the third bulb when lower boiling material will distil into the fourth bulb. The process is continued until no more material distils into the subsequent bulb. The vacuum (if applied) and the furnace are removed, the bulbs are separated and the various fractions of distillates are collected from the individual bulbs. This procedure is used for preliminary purification and the distillates are then redistilled or recrystallised.

Vacuum-lines, Schlenk and Glovebox Techniques. Manipulations involving materials sensitive to air or water vapour can be carried out by these procedures. Vacuum-line methods make use of quantitative transfers, and **P**(pressure)-**V**(volume)-**T**(temperature) measurements, of gases, and trap-to-trap separations of volatile substances.
It is usually more convenient to work under an inert-gas atmosphere, using **Schlenk** type apparatus. The *principle* of Schlenk methods is the bottle which has a standard ground-glass joint and a sidearm with a tap. The system can be purged by evacuating and flushing with an inert gas (usually nitrogen, or in some cases, argon), repeating the process until the contaminants in the vapour phases have been diminished to acceptable limits. If the bottom of the bottle has a tap and a cone, a dropping bottle is produced, while further addition of a sinter disk in the bottle converts it to a filter funnel. With these, and tailor-made pieces of glassware, inert atmospheres can be maintained during crystallisation, filtration, sublimation and transfer. Schlenk-type glassware is commercially available (as *Airless Ware*) from Kontes Glass Co, Vineland, NJ, USA and Embell Scientific, Murwillumbah, NSW 2484, Australia).
Syringe techniques have been worked out for small volumes, while for large volumes or where much manipulation is required, dryboxes (*glove boxes*) or dry chambers should be used.
For fuller discussion, see Sanderson *Vacuum Manipulation of Volatile Compounds* John Wiley and Sons Ltd, NY, 1948; L.W.Muller *Vacuum Technology: Principles and Applications*, Chapman & Hall Ltd, 1995; W.H.Kohl *Handbook of Materials & Techniques for Vacuum Devices*, American Institute of Physics Press, 1994; Shriver *The Manipulation of Air-sensitive Compounds* McGraw-Hill Book Co, NY, 1969; Brown *Organic Syntheses via Boranes,* Wiley, NY, 1975; A.Pelter *Borane Reagents*, Academic Press Inc., 1988.

Spinning-band Columns. Factors which limit the performance of distillation columns include the tendency to flood (which occurs when the returning liquid blocks the pathway taken by the vapour through the column) and the increased hold-up (which decreases the attainable efficiency) in the column that should, theoretically, be highly efficient. To overcome these difficulties, especially for distillation under high vacuum of heat sensitive or high-boiling highly viscous fluids, spinning band columns have become commercially available. In such units, the distillation columns contain a rapidly rotating, motor-driven, spiral band, which may be of polymer-coated metal, stainless steel or platinum. The rapid rotation of the band in contact with the walls of the still gives intimate mixing of descending liquid and ascending vapour while the screw-like motion of the band drives the liquid towards the still-pot, helping to reduce hold-up. There is very little pressure drop in such a system, and very high throughputs are possible, at high efficiency. For example, a 30-in 10-mm diameter commercial column is reported to have an efficiency of 28 plates and a pressure drop of 0.2mm Hg for a throughput of 330ml/h. The columns may be either vacuum jacketed or heated externally. The stills can be operated down to 10^{-5}mm Hg. The principle, which was first used commercially in the Podbielniak Centrifugal Superfractionator, has also been embodied in descending-film molecular distillation apparatus.

STEAM DISTILLATION

When two immmiscible liquids distil, the sum of their (independent) partial pressures is equal to the atmospheric pressure. Hence in steam distillation, the distillate has the composition

$$\frac{\text{Moles of substance}}{\text{Moles of water}} = \frac{P_{substance}}{P_{water}} = \frac{760 - P_{water}}{P_{water}}$$

where the *P*'s are vapour pressures in mm Hg) in the boiling mixture. One of the advantages of using water in this way lies in its low molecular weight.
The customary technique consists of heating the substance and water in a flask (to boiling), usually with the passage of steam, followed by condensation and separation of the aqueous and non-aqueous phases. Its advantages are those of selectivity (because only some water-insoluble substances, such as naphthalene, nitrobenzene, phenol and aniline are volatile in steam) and of ability to distil certain high-boiling substances well below their boiling point. It also facilitates the recovery of a non-steam-volatile solid at a relatively low temperature from a high-boiling solvent such as nitrobenzene. The efficiency of steam distillation is increased if superheated steam is used (because the vapour pressure of the organic component is increased relative to water). In this case the flask containing the material is heated (without water) in an oil bath and the steam passing through it is superheated by prior passage through a suitable heating device (such as a copper coil over a bunsen burner or an oil bath). (For further detail, see Krell 1963, p 45).

AZEOTROPIC DISTILLATION

In some cases two or more liquids form constant-boiling mixtures, or azeotropes. Azeotropic mixtures are most likely to be found with components which readily form hydrogen bonds or are otherwise highly associated, especially when the components are dissimilar, for example an alcohol and an aromatic hydrocarbon, but have similar boiling points. (Many systems are summarised in *Azeotropic Data - III*, L.H.Horsley, *Advances in Chemistry Series 116*, American Chemical Society, Washington, *1973*).

Examples where the boiling point of the distillate is a minimum (less than either pure component) include:
Water with ethanol, *n*-propanol and isopropanol, *tert*-butanol, propionic acid, butyric acid, pyridine,
methanol with methyl iodide, methyl acetate, chloroform,
ethanol with ethyl iodide, ethyl acetate, chloroform, benzene, toluene, methyl ethyl ketone,
benzene with cyclohexane,
acetic acid with toluene.
Although less common, azeotropic mixtures are known which have higher boiling points than their components. These include water with most of the mineral acids (hydrofluoric, hydrochloric, hydrobromic, perchloric, nitric and sulphuric) and formic acid. Other examples are acetic acid-pyridine, acetone-chloroform, aniline-phenol, and chloroform-methyl acetate.

The following azeotropes are important commercially for drying ethanol:

ethanol 95.5% (by weight) - water 4.5%	**b** 78.1°
ethanol 32.4% - benzene 67.6%	**b** 68.2°
ethanol 18.5% - benzene 74.1% - water 7.4%	**b** 64.9°

Materials are sometimes added to form an azeotropic mixture with the substance to be purified. Because the azeotrope boils at a different temperature, this facilitates separation from substances distilling in the same range as the pure material. (Conversely, the impurity might form the azeotrope and be removed in this way). This method is often convenient, especially where the impurities are isomers or are otherwise closely related to the desired substance. Formation of low-boiling azeotropes also facilitates distillation.

One or more of the following methods can generally be used for separating the components of an azeotropic mixture:

1. By using a chemical method to remove most of one species prior to distillation. (For example, water can be removed by suitable drying agents; aromatic and unsaturated hydrocarbons can be removed by sulphonation).
2. By redistillation with an additional substance which can form a ternary azeotropic mixture (as in ethanol-water-benzene example given above).
3. By selective adsorption of one of the components. (For example , of water on to a silica gel or molecular sieve, or of unsaturated hydrocarbons on to alumina).
4. By fractional crystallisation of the mixture, either by direct freezing or after solution in a suitable solvent.

ISOPIESTIC OR ISOTHERMAL DISTILLATION

This technique can be useful for the preparation of metal-free solutions of volatile acids and bases for use in trace metal studies. The procedure involves placing two beakers, one of distilled water and the other of a solution of

the material to be purified, in a desiccator. The desiccator is sealed and left to stand at room temperature for several days. The volatile components distribute themselves between the two beakers whereas the non-volatile contaminants remain in the original beaker. This technique has afforded metal-free pure solutions of ammonia, hydrochloric acid and hydrogen fluoride.

SUBLIMATION

Sublimation differs from ordinary distillation because the vapour condenses to a solid instead of a liquid. Usually, the pressure in the heated system is diminished by pumping, and the vapour is condensed (after travelling a relatively short distance) on to a cold finger or some other cooled surface. This technique, which is applicable to many organic solids, can also be used with inorganic solids such as aluminium chloride, ammonium chloride, arsenious oxide and iodine. In some cases, passage of a stream of inert gas over the heated substance secures adequate vaporisation.

RECRYSTALLISATION

Techniques

The most commonly used procedure for the purification of a solid material by recrystallisation from a solution involves the following steps:

(a) The impure material is dissolved in a suitable solvent, by shaking or vigorous stirring, at or near the boiling point, to form a near-saturated solution.
(b) The hot solution is filtered to remove any insoluble particles. To prevent crystallisation during this filtration, a heated (jacketed) filter funnel can be used or the solution can be somewhat diluted with more of the solvent.
(c) The solution is then allowed to cool so that the dissolved substance crystallises out.
(d) The crystals are separated from the mother liquor, either by centrifuging or by filtering, under suction, through a sintered glass, a Hirsch or a Büchner, funnel. Usually, centrifuging is much preferred because of the much greater ease and efficiency of separating crystals and mother liquor, and also because of the saving of time and effort, particularly when very small crystals are formed or when there is entrainment of solvent.
(e) The crystals are washed free from mother liquor with a little fresh cold solvent, then dried.

If the solution contains extraneous coloured material likely to contaminate the crystals, this can often be removed by adding some activated charcoal (decolorising carbon) to the hot, but not boiling, solution which is then shaken frequently for several minutes before being filtered. (The large active surface of the carbon makes it a good adsorbent for this purpose.) In general, the cooling and crystallisation step should be rapid so as to give small crystals which occlude less of the mother liquor. This is usually satisfactory with inorganic material, so that commonly the filtrate is cooled in an ice-water bath while being vigorously stirred. In many cases, however, organic molecules crystallise much more slowly, so that the filtrate must be set aside to cool to room temperature or left in the refrigerator. It is often desirable to subject material that is very impure to preliminary purification, such as steam distillation, Soxhlet extraction, or sublimation, before recrystallising it. A greater degree of purity is also to be expected if the crystallisation process is repeated several times, especially if different solvents are used. The advantage of several crystallisations from different solvents lies in the fact that the material sought, and its impurities, are unlikely to have similar solubilities as solvents and temperatures are varied.
For the final separation of solid material, sintered-glass discs are preferable to filter paper. Sintered glass is unaffected by strongly acid solutions or by oxidising agents. Also, with filter paper, cellulose fibres are likely to become included in the sample, The sintered-glass discs or funnels can be readily cleaned by washing in freshly prepared *chromic acid cleaning mixture*. This mixture is made by adding 100ml of concentrated sulphuric acid slowly with stirring to a solution of 5g of sodium dichromate in 5ml of water. (The mixture warms to about 70°).
For materials with melting points below 70° it is sometimes convenient to use dilute solutions in acetone, methanol, pentane, ethyl ether or $CHCl_3$-CCl_4. The solutions are cooled to -78° in Dry-ice, to give a filtrable slurry which is filtered off through a precooled Büchner funnel. Experimental details, as applied to the purification of nitromethane, are given by Parrett and Sun [*J Chem Educ* **54** 448 *1977*].
Where substances vary little in solubility with temperature, isothermal crystallisation may sometimes be employed. This usually takes the form of a partial evaporation of a saturated solution at room temperature by leaving it under reduced pressure in a desiccator.
However, in rare cases, crystallisation is not a satisfactory method of purification, especially if the impurity forms crystals that are isomorphous with the material being purified. In fact, the impurity content may even be greater in

such recrystallised material. For this reason, it still remains necessary to test for impurities and to remove or adequately lower their concentrations by suitable chemical manipulation prior to recrystallisation.

Filtration

Filtration removes particulate impurities rapidly from liquids and is also used to collect insoluble or crystalline solids which separate or crystallise from solution. The usual technique is to pass the solution, cold or hot, through a fluted filter paper in a conical glass funnel (see Vogel's *Textbook of Practical Organic Chemistry*, p 46).

If a solution is hot and needs to be filtered rapidly a Büchner funnel and flask are used and filtration is performed under a slight vacuum (water pump), the filter medium being a circular cellulose filter paper wet with solvent. If filtration is slow, even under high vacuum, a pile of about twenty filter papers, wet as before, are placed in the Büchner funnel and, as the flow of solution slows down, the upper layers of the filter paper are progressively removed. Alternatively, a filter aid, e.g. Celite, Florisil or Hyflo-supercel, is placed on top of a filter paper in the funnel. When the flow of the solution (under suction) slows down the upper surface of the filter aid is scratched gently. Filter papers with various pore sizes are available covering a range of filtration rates. Hardened filter papers are slow filtering but they can withstand acidic and alkaline solutions without appreciable hydrolysis of the cellulose (see Table 3). When using strong acids it is preferable to use glass micro fibre filters which are commercially available (see Table 3).

Freeing a solution from extremely small particles (e.g. for ORD or CD measurements) requires filters with very small pore size. Commercially available (Millipore, Gelman, Nucleopore) filters other than cellulose or glass include nylon, Teflon, and polyvinyl chloride, and the pore diameter may be as small as 0.01micron (see Table 4). Special containers are used to hold the filters, through which the solution is pressed by applying pressure, e.g. from a syringe. Some of these filters can be used to clear strong sulphuric acid solutions .
As an alternative to the Büchner funnel for collecting crystalline solids, a funnel with a sintered glass-plate under suction may be used. Sintered-glass funnels with various porosities are commercially available and can easily cleaned with warm chromic or nitric acid (see above).

When the solid particles are too fine to be collected on a filter funnel because filtration is extremely slow, separation by **centrifugation** should be used. Bench type centrifuges are most convenient for this purpose. The solid is placed in the centrifuge tube, the tubes containing the solutions on opposite sides of the rotor should be balanced accurately (at least within 0.05 to 0.1g), and the solutions are spun at maximum speed for as long as it takes to settle the solid (usually *ca* 3-5 minutes). The solid is washed with cold solvent by centrifugation, and finally twice with a pure volatile solvent in which the solid is insoluble, also by centrifugation. After decanting the supernatant the residue is dried in a vacuum, at elevated temperatures if necessary. In order to avoid "spitting" and contamination with dust while the solid in the centrifuge tube is dried, the mouth of the tube is covered with silver paper and held fast with a tight rubber band near the lip. The flat surface of the silver paper is then perforated in several places with a pin.

Choice of Solvents

The best solvents for recrystallisation have the following properties:

(a) The material is much more soluble at higher temperatures than it is at room temperature or below.
(b) Well-formed (but not large) crystals are produced.
(c) Impurities are either very soluble or only sparingly soluble.
(d) The solvent must be readily removed from the purified material.
(e) There must be no reaction between the solvent and the substance being purified.
(f) The solvent must not be inconveniently volatile or too highly flammable. (These are reasons why ethyl ether and carbon disulphide are not commonly used in this way.)

The following generalisations provide a rough guide to the selection of a suitable solvent:

(a) Substances usually dissolve best in solvents to which they are most closely related in chemical and physical characteristics. Thus, hydroxylic compounds are likely to be most soluble in water, methanol, ethanol, acetic acid or acetone. Similarly, petroleum ether might be used with water-insoluble substances. However, if the resemblance is too close, solubilities may become excessive.
(b) Higher members of homologous series approximate more and more closely to their parent hydrocarbon.
(c) Polar substances are more soluble in polar, than in non-polar, solvents.

Although Chapters 3, 4 and 5 provide details of the solvents used for recrystallising a large portion of commercially available laboratory chemicals, they cannot hope to be exhaustive, nor need they necessarily be the best choice. In other cases where it is desirable to use this process, it is necessary to establish whether a given solvent is suitable. This is usually done by taking only a small amount of material in a small test-tube and adding enough solvent to cover it. If it dissolves readily in the cold or on gentle warming, the solvent is unsuitable. Conversely, if it remains insoluble when the solvent is heated to boiling (adding more solvent if necessary), the solvent is again unsuitable. If the material dissolves in the hot solvent but does not crystallise readily within several minutes of cooling in an ice-salt mixture, another solvent should be tried.
Solvents commonly used for recrystallisation, and their boiling points, are given in Table 5.

Mixed Solvents
Where a substance is too soluble in one solvent and too insoluble in another, for either to be used for recrystallisation, it is often possible (provided they are miscible) to use them as a mixed solvent. (In general, however, it is preferable to use a single solvent if this is practicable.) Table 6 contains many of the common pairs of miscible solvents.
The technique of recrystallisation from a mixed solvent is as follows:
The material is dissolved in the solvent in which it is the more soluble, then the other solvent (heated to near boiling) is added cautiously to the hot solution until a slight turbidity persists or crystallisation begins. This is cleared by adding several drops of the first solvent, and the solution is allowed to cool and crystallise in the usual way.
A variation of this procedure is simply to precipitate the material in a microcrystalline form from solution in one solvent at room temperature, by adding a little more of the second solvent, filtering this off, adding a little more of the second solvent and repeating the process. This ensures, at least in the first or last precipitation, a material which contains as little as possible of the impurities which may also be precipitated in this way. With salts the first solvent is commonly water, and the second solvent is alcohol or acetone.

Recrystallisation from the Melt
A crystalline solid melts when its temperature is raised sufficiently for the thermal agitation of its molecules or ions to overcome the restraints imposed by the crystal lattice. Usually, impurities weaken crystal structures, and hence lower the melting points of solids (or the freezing points of liquids). If an impure material is melted and cooled slowly (with the addition, if necessary, of a trace of solid material near the freezing point to avoid supercooling), the first crystals that form will usually contain less of the impurity, so that fractional solidification by partial freezing can be used as a purification process for solids with melting points lying in a convenient temperature range (or for more readily frozen liquids). In some cases, impurities form higher melting eutectics with substances to be purified, so that the first material to solidify is less pure than the melt. For this reason, it is often desirable to discard the first crystals and also the final portions of the melt. Substances having similar boiling points often differ much more in melting points, so that fractional solidification can offer real advantages, especially where ultrapurity is sought.
The technique of recrystallisation from the melt as a means of purification dates back from its use by Schwab and Wichers (*J Res Nat Bur Stand* **25** 747 *1940*) to purify benzoic acid. It works best if material is already nearly pure, and hence tends to be a final purification step. A simple apparatus for purifying organic compounds by progressive freezing is described by Matthias and Coggeshall (*AC* **31** 1124 *1959*). In principle, the molten substance is cooled slowly by progressive lowering of the tube containing it into a suitable bath. For temperatures between 0° and 100°, waterbaths are convenient. Where lower temperatures are required, the cooling baths given in Table 7 can be used. Cooling is stopped when part of the melt has solidified, and the liquid phase is drained off. Column crystallisation has been used to purify stearyl alcohol, cetyl alcohol, myristic acid; fluorene, phenanthrene, biphenyl, terphenyls, dibenzyl; phenol, 2-naphthol; benzophenone and 2,4-dinitrotoluene; and many other organic (and inorganic) compounds. [See, for example, *Developments in Separation Science* N.N.Lee (ed), CRC Press, Cleveland, Ohio, *1972*]. Thus, an increase in purity from 99.80 to 99.98 mole% was obtained when acetamide was slowly crystallised in an insulated round bottom flask until half the material had solidified and the solid phase was then recrystallised from benzene [Schwab and Wichers *J Res Nat Bur Stand* **32** 253 *1944*].
Fractional solidification and its applications to obtaining ultrapure chemical substances, has been treated in detail in *Fractional Solidification* by M.Zief and W.R.Wilcox eds, Edward Arnold Inc, London *1967*, and *Purification of Inorganic and Organic Materials* by M.Zief, Marcel Dekker Inc, New York *1969*. These monographs should be consulted for discussion of the basic principles of solid-liquid processes such as zone melting, progressive freezing and column crystallisation, laboratory apparatus and industrial scale equipment, and examples of applications. These include the removal of cyclohexane from benzene, and the purification of aromatic amines, dienes and naphthalene,

and inorganic species such as the alkali iodides, potassium chloride, indium antimonide and gallium trichloride. The authors also discuss analytical methods for assessing the purity of the final material.

Zone Refining

Zone refining (or zone melting) is a particular development for fractional solidification and is applicable to all crystalline substances that show differences in soluble impurity concentration in liquid and solid states at solidification. The apparatus used in this technique consists essentially of a device by which a narrow molten zone moves slowly down a long tube filled with the material to be purified. The machine can be set to recycle repeatedly. At its advancing side, the zone has a melting interface with the impure material whereas on the upper surface of the zone there is a constantly growing face of higher-melting, resolidified material. This leads to a progressive increase in impurity in the liquid phase which, at the end of the run, is discarded. Also, because of the progressive increase in impurity in the liquid phase, the resolidified material becomes correspondingly less further purified. For this reason, it is usually necessary to make several zone-melting runs before a sample is satisfactorily purified. This is also why the method works most successfully if the material is already fairly pure. In all these operations the zone must travel slowly enough to enable impurities to diffuse or be convected away from the area where resolidification is occurring.

The technique finds commercial application in the production of metals of extremely high purity (impurities down to 10^{-9} ppm), in purifying refractory oxides, and in purifying organic compounds, using commercially available equipment. Criteria for indicating that definite purification is achieved include elevation of melting point, removal of colour, fluorescence or smell, and a lowering of electrical conductivity. Difficulties likely to be met with in organic compounds, especially those of low melting points and low rates of crystallisation, are supercooling and, because of surface tension and contraction, the tendency of the molten zone to seep back into the recrystallised areas. The method is likely to be useful in cases where fractional distillation is not practicable, either because of unfavourable vapour pressures or ease of decomposition, or where super-pure materials are required. It has been used for the latter purpose with anthracene, benzoic acid, chrysene, morphine and pyrene. (See references on p. 47).

DRYING

Removal of Solvents

Where substances are sufficiently stable, removal of solvent from recrystallised materials presents no problems. The crystals, after filtering at the pump (and perhaps air-drying by suction), are heated in an oven above the boiling point of the solvent (but below their melting point), followed by cooling in a desiccator. Where this treatment is inadvisable, it is still often possible to heat to a lower temperature under reduced pressure, for example in an Abderhalden pistol. This device consists of a small chamber which is heated externally by the vapour of a boiling solvent. Inside this chamber, which can be evacuated by a water pump or some other vacuum pump, is placed a small boat containing the sample to be dried and also a receptacle with a suitable drying agent. Convenient liquids for use as boiling liquids in an Abderhalden pistol, and their temperatures, are given in Table 9. In cases where heating above room temperature cannot be used, drying must be carried out in a vacuum desiccator containing suitable absorbants. For example, hydrocarbons, such as benzene, cyclohexane and petroleum ether, can be removed by using shredded paraffin wax, and acetic acid and other acids can be absorbed by pellets of sodium, or potassium, hydroxide. However, in general, solvent removal is less of a problem than ensuring that the water content of solids and liquids is reduced below an acceptable level.

Removal of Water

Methods for removing water from solids depends on the thermal stability of the solids or the time available. The safest way is to dry in a vacuum desiccator over concentrated sulphuric acid, phosphorus pentoxide, silica gel, calcium chloride, or some other desiccant. Where substances are stable in air and melt above 100° drying in an air oven may be adequate. In other cases, use of an Abderhalden pistol may be satisfactory.

Often, in drying inorganic salts, the final material that is required is a hydrate. In such cases, the purified substance is left in a desiccator to equilibrate above an aqueous solution having a suitable water-vapour pressure. A convenient range of solutions used in this way is given in Table 10.

The choice of desiccants for drying liquids is more restricted because of the need to avoid all substances likely to react with the liquids themselves. In some cases, direct distillation of an organic liquid is a suitable method for drying both solids and liquids, especially if low-boiling azeotropes are formed. Examples include acetone, aniline, benzene, chloroform, carbon tetrachloride, ethylene dichloride, heptane, hexane, methanol, nitrobenzene, petroleum ether, toluene and xylene. Addition of benzene can be used for drying ethanol by distillation. In carrying out distillations intended to yield anhydrous products, the apparatus should be fitted with guard-tubes containing calcium chloride or silica gel to prevent entry of moist air into the system. (Many anhydrous organic liquids are appreciably hygroscopic).

Traces of water can be removed from solvents such as benzene, 1,2-dimethoxyethane, ethyl ether, CH_2Cl_2, pentane, toluene and tetrahydrofuran by refluxing under nitrogen a solution containing **sodium benzophenone ketyl**, and fractionally distilling. Drying with, and distilling from CaH_2 is applicable to a number of solvents including aniline, benzene, *tert*-butylamine, *tert*-butanol, 2,4,6-collidine, diisopropylamine, dimethylformamide, hexamethylphosphoramide, methylenedichloride, pyridine, tetramethylethylenediamine, toluene, triethylamine.

Removal of water from gases may be by physical or chemical means, and is commonly by adsorption on to a drying agent in a low-temperature trap. The effectiveness of drying agents depends on the vapour pressure of the hydrated compound - the lower the vapour pressure the less the remaining moisture in the gas.
The most usually applicable of the specific methods for detecting and determining water in organic liquids is due to Karl Fischer. (See J.Mitchell and D.M.Smith, *Aquametry*, Interscience, New York, *1948*; Fieser and Fieser *Reagents for Organic Synthesis*, J.Wiley & Sons, NY, Vol 1, 528 *1967*). Other techniques include electrical conductivity measurements and observation of the temperature at which the first cloudiness appears as the liquid is cooled (applicable to liquids in which water is only slightly soluble). Addition of anhydrous cobalt (II) iodide (blue) provides a convenient method (colour change to pink on hydration) for detecting water in alcohols, ketones, nitriles and some esters. Infrared absorption measurements of the broad band for water near 3500 cm^{-1} can also sometimes be used for detecting water in non-hydroxylic substances.

Intensity and Capacity of Common Desiccants

Drying agents can be conveniently be grouped into three classes, depending on whether they combine with water reversibly, they react chemically (irreversibly) with water, or they are molecular sieves. The first group vary in their drying intensity with the temperature at which they are used, depending on the vapour pressure of the hydrate that is formed. This is why, for example, drying agents such as anhydrous sodium sulphate, magnesium sulphate or calcium chloride should be filtered off from the liquids before the latter are heated. The intensities of drying agents belonging to this group fall in the sequence:

P_2O_5 >> BaO > $Mg(ClO_4)_2$, CaO, MgO, KOH (fused), conc H_2SO_4, $CaSO_4$, Al_2O_3 > KOH (sticks), silica gel, $Mg(ClO_4)_2.3H_2O$ > NaOH (fused), 95% H_2SO_4, $CaBr_2$, $CaCl_2$ (fused) > NaOH (sticks), $Ba(ClO_4)_2$, $ZnCl_2$ (sticks), $ZnBr_2$ > $CaCl_2$ (technical) > $CuSO_4$ > Na_2SO_4, K_2CO_3.

Where large amounts of water are to be removed, a preliminary drying of liquids is often possible by shaking with concentrated solutions of calcium chloride or potassium carbonate, or by adding sodium chloride to salt out the organic phase (for example, in the drying of lower alcohols).
Drying agents that combine irreversibly with water include the alkali metals, the metal hydrides (discussed in Chapter 2), and calcium carbide.

Suitability of Individual Desiccants

Alumina. (Preheated to 175° for about 7h). Mainly as a drying agent in a desiccator or as a column through which liquid is percolated.

Aluminium amalgam. Mainly used for removing traces of water from alcohols, which are distilled from it after refluxing.

Barium oxide. Suitable for drying organic bases.

Barium perchlorate. Expensive. Used in desiccators (*covered with a metal guard*). Unsuitable for drying solvents or organic material where contact is necessary, because of the danger of **EXPLOSION**

Boric anhydride. (Prepared by melting boric acid in an air oven at a high temperature, cooling in a desiccator, and powdering.) Mainly used for drying formic acid.

Calcium chloride (anhydrous). Cheap. Large capacity for absorption of water, giving the hexahydrate below 30°, but is fairly slow in action and not very efficient. Its main use is for preliminary drying of alkyl and aryl halides, most esters, saturated and aromatic hydrocarbons and ethers. Unsuitable for drying alcohols and amines (which form addition compounds), fatty acids, amides, amino acids, ketones, phenols, or some aldehydes and esters. Calcium chloride is suitable for drying the following gases: hydrogen, hydrogen chloride, carbon monoxide, carbon dioxide, sulphur dioxide, nitrogen, methane, oxygen, also paraffins, ethers, olefines and alkyl chlorides.

Calcium hydride. See Chapter 2.

Calcium oxide. (Preheated to 700-900° before use.) Suitable for alcohols and amines (but does not dry them completely). Need not be removed before distillation, but in that case the head of the distillation column should be packed with glass wool to trap any calcium oxide powder that might be carried over. Unsuitable for acidic compounds and esters. Suitable for drying gaseous amines and ammonia.

Calcium sulphate (anhydrous). (Prepared by heating the dihydrate or the hemihydrate in an oven at 235° for 2-3h; it can be regenerated.) Available commercially as Drierite. It forms the hemihydrate, $2CaSO_4.H_2O$, so that its capacity is fairly low (6.6% of its weight of water), and hence is best used on partially dried substances. It is very efficient (being comparable with phosphorus pentoxide and concentrated sulphuric acid). Suitable for most organic compounds. Solvents boiling below 100° can be dried by direct distillation from calcium sulphate.

Copper (II) sulphate (anhydrous). Suitable for esters and alcohols. Preferable to sodium sulphate in cases where solvents are sparingly soluble in water (for example, benzene or toluene).

Lithium aluminium hydride. See Chapter 2.

Magnesium amalgam. Mainly used for removing traces of water from alcohols, which are distilled from it after refluxing.

Magnesium perchlorate (anhydrous). (Available commercially as Dehydrite. Expensive.) Used in desiccators. Unsuitable for drying solvents or any organic material where contact is necessary, because of the **danger of EXPLOSION**.

Magnesium sulphate (anhydrous). (Prepared from the heptahydrate by drying at 300° under reduced pressure.) More rapid and effective than sodium sulphate. It has a large capacity, forming $MgSO_4.7H_2O$ below 48°. Suitable for the preliminary drying of most organic compounds.

Molecular sieves. See page 28.

Phosphorus pentoxide. Very rapid and efficient, but difficult to handle and should only be used after the organic material has been partially dried, for example with magnesium sulphate. Suitable for acid anhydrides, alkyl and aryl halides, ethers, esters, hydrocarbons and nitriles, and for use in desiccators. Not suitable with acids, alcohols, amines or ketones, or with organic molecules from which a molecule of water can be fairly readily abstracted by an elimination rection. Suitable for drying the following gases: hydrogen, oxygen, carbon dioxide, carbon monoxide, sulphur dioxide, nitrogen, methane, ethylene and paraffins. It is available with an indicator (cobalt salt, blue when dry and pink when wet) under the name *Sicapent* (from Merck).

Potassium (metal). Properties and applications are similar to those for sodium, and it is a correspondingly hazardous substance.

Potassium carbonate (anhydrous). Has a moderate efficiency and capacity, forming the dihydrate. Suitable for an initial drying of alcohols, bases, esters, ketones and nitriles by shaking with them, then filtering off. Also suitable for salting out water-soluble alcohols, amines and ketones. Unsuitable for acids, phenols and other acidic substances.

Potassium hydroxide. Solid potassium hydroxide is very rapid and efficient. Its use is limited almost entirely to the initial drying of organic bases. Alternatively, sometimes the base is shaken first with a concentrated solution of potassium hydroxide to remove most of the water present. Unsuitable for acids, aldehydes, ketones, phenols, amides and esters. Also used for drying gaseous amines and ammonia.

Silica gel. Granulated silica gel is a commercially available drying agent for use with gases, in desiccators, and (because of its chemical inertness) in physical instruments (pH meters, spectrometers, balances). Its drying action depends on physical adsorption, so that silica gel must be used at room temperature or below. By incorporating cobalt chloride into the material it can be made self indicating, re-drying in an oven at 110° being necessary when the colour changes from blue to pink.

Sodium (metal). Used as a fine wire or as chips, for more completely drying ethers, saturated hydrocarbons and aromatic hydrocarbons which have been partially dried (for example with calcium chloride or magnesium sulphate). Unsuitable for acids, alcohols, alkyl halides, aldehydes, ketones, amines and esters. Reacts violently if much water is present and can cause a fire with highly flammable liquids.

Sodium hydroxide. Properties and applications are similar to those for potassium hydroxide.

Sodium-potassium alloy. Used as lumps. Lower melting than sodium, so that its surface is readily renewed by shaking. Properties and applications are similar to those for sodium.

Sodium sulphate (anhydrous). Has a large capacity for absorption of water, forming the decahydrate below 33°, but drying is slow and inefficient, especially for solvents that are sparingly soluble in water. It is suitable for the preliminary drying of most types of organic compounds.

Sulphuric acid (concentrated). Widely used in desiccators. Suitable for drying bromine, saturated hydrocarbons, alkyl and aryl halides. Also suitable for drying the following gases: hydrogen, nitrogen, carbon dioxide, carbon monoxide, chlorine, methane and paraffins. Unsuitable for alcohols, bases, ketones or phenols. Also available with an indicator (a cobalt salt, blue when dry and pink when wet) under the name *Sicacide* (from Merck) for desiccators.

For convenience, many of the above drying agents are listed in Table 11 under the classes of organic compounds for which they are commonly used.

Freeze-pump-thaw and Purging

Volatile contaminants, e,g, traces of low boiling solvent residue or oxygen, in liquid samples or solutions can be very deleterious to the samples on storage. These contaminants can be removed by repeated freeze-pump-thaw cycles. This involves freezing the liquid material under high vacuum in an appropriate vessel (which should be large enough to avoid contaminating the vacuum line with liquid that has bumped) connected to the vacuum line *via* efficient liquid nitrogen traps. The frozen sample is then thawed until it liquefies, kept in this form for some time (*ca*, 10-15min), refreezing the sample and the cycle repeated several times without interupting the vacuum. This procedure applies equally well to solutions, as well as purified liquids, e.g. as a means of removing oxygen from solutions for NMR and other measurements. If the presence of nitrogen, helium or argon, is not a serious contaminant then solutions can be freed from gases, e.g. oxygen, carbon dioxide, and volatile impurities by purging with N_2, He or Ar at room, or slightly elevated, temperature. The gases used for purging are then removed by freeze-pump-thaw cycles or simply by keeping in a vacuum for several hours.

CHROMATOGRAPHY

Chromatography is often used with advantage for the purification of small amounts of complex organic mixtures, either as liquid chromatography or as vapour phase (gas) chromatography.

Liquid Chromatography

The mobile phase in liquid chromatography is a liquid and the stationary phase is of four main types. These are for adsorption, partition, ion-chromatography, and gel filtration. The technique of chromatography which applies to all liquid chromatography at atmospheric pressure comprises the following distinct steps. The material is adsorbed as a level bed onto the column of stationary phase. (It is important that this bed is as narrow as possible because the bands of components in the mixture that is applied widen as they move with the mobile phase down the column.) The column is washed (developed) with a quantity of pure solvent or solvent mixture. The column may be pushed out of the tube so that it can be divided into zones. The desired components are then extracted from the appropriate zones using a suitable solvent. Alternatively, and more commonly, the column is left intact and the bands are progressively eluted by passing more solvent through the column.

Adsorption Chromatography

Adsorption chromatography is based on the difference in the extent to which substances in solution are adsorbed onto a suitable surface. The substances to be purified are usually placed on the top of the column and the solvent is run down the column. In a more common variation of this method, the column containing the adsorbent is full of solvent before applying the mixture at the top of the column. In another application the mixture is adsorbed onto a small amount of stationary phase and placed at the bottom of the column with the dry stationary phase above it. By applying a slight vacuum at the top of the column, the eluting solvent can be sucked slowly upwards from the bottom of the column. When the solvent has reached the top of the column the separation is complete and the vacuum is released. The packing is pushed gently out of the tube and cut into strips as above. Alternatively the vacuum is kept and the effluent from the top of the column is collected in fractions. The fractions are monitored by UV or visible spectra, colour reactions or other means for identifying the components.

Graded Adsorbents and Solvents. Materials used in columns for adsorption chromatography are grouped in Table 12 in an approximate order of effectiveness. Other adsorbents sometimes used include barium carbonate, calcium sulphate, charcoal (usually mixed with kieselguhr or other form of diatomaceous earth, for example, the filter aid Celite), cellulose, glucose and lactose. The alumina can be prepared in several grades of activity (see below).

In most cases, adsorption takes place most readily from non-polar solvents, such as petroleum ether or benzene, and least readily from polar solvents such as alcohols, esters, and acetic acid. Common solvents, arranged in approximate order of increasing eluting ability are also given in Table 12.

Eluting power roughly parallels the dielectric constants of solvents. The series also reflects the extent to which the solvent binds to the column material, thereby displacing the substances that are already adsorbed. This preference of alumina and silica gel for polar molecules explains, for example, the use of percolation through a column of silica gel for the following purposes-drying of ethylbenzene, removal of aromatics from 2,4-dimethylpentane and of ultraviolet absorbing substances from cyclohexane.

Mixed solvents are intermediate in strength, and so provide a finely graded series. In choosing a solvent for use as an eluent it is necessary to consider the solubility of the substance in it, and the ease with which it can subsequently be removed.

Preparation and Standardisation of Alumina. The activity of alumina depends inversely on its water content, and a sample of poorly active material can be rendered more active by leaving for some time in a round bottomed flask heated up to about 200° in an oil bath or a heating mantle while a slow stream of a dry inert gas is passed through it. Alternatively, it is heated to red heat (380-400°) in an open vessel for 4-6h with occasional stirring and then cooled in a vacuum desiccator: this material is then of grade I activity. Conversely, alumina can be rendered less active by adding small amounts of water and thoroughly mixing for several hours. Addition of about 3% (w/w) of water converts grade I alumina to grade II.

Used alumina can be regenerated by repeated extraction, first with boiling methanol, then with boiling water, followed by drying and heating. The degree of activity of the material can be expressed conveniently in terms of the scale due to Brockmann and Schodder (*B* B **74** 73 *1941*). This system is based on the extent of adsorption of five pairs of azo dyestuffs, being adjacent members of the set: azobenzene, *p*-methoxyazobenzene, Sudan yellow, Sudan red, aminoazobenzene, hydroxyazobenzene. In testing the alumina, a tube 10cm long by 1.5cm internal diameter is packed with alumina to a depth of 5cm and covered with a disc of filter paper. The dyestuff solutions are prepared by dissolving 2mg of each azo dye of the pair in 2ml of purified benzene (distilled from potassium hydroxide) and 8ml of

petroleum ether. The solution is applied to the column and developed with 20ml of benzene-petroleum ether mixture (1:4 v/v) at a flow rate of about 20-30 drops per min. The behaviour in the following Table is observed:

GRADE	POSITION OF ZONES (a)	(b)	(c)
I	*p*-Methoxyazobenzene	Azobenzene	
II	*p*-Methoxyazobenzene(d)	Azobenzene	
II	Sudan Yellow	*p*-Methoxyazobenzene	
III		Sudan Yellow	*p*-Methoxy-azobenzene
III	Sudan Red	Sudan Yellow	
IV	Sudan Red		Sudan Yellow
IV	Aminoazobenzene	Sudan Red	
V	Hydroxyazobenzene	Aminoazobenzene	

(a) Near top of column. (b) Near bottom of column. (c) In effluent. (d) 1 to 2 cm from top. Grade I is most active, Grade V is least active.

Alumina is normally slightly alkaline. A (less strongly adsorbing) neutral alumina can be prepared by making a slurry in water and adding 2M hydrochloric acid until the solution is acid to Congo red. The alumina is then filtered off, washed with distilled water until the wash water gives only a weak violet colour with Congo red paper, and dried.
Alumina used in TLC can be recovered by washing in ethanol for 48h with occasional stirring, to remove binder material and then washed with successive portions of ethyl acetate, acetone and finally with distilled water. Fine particles are removed by siphoning. The alumina is first suspended in 0.04M acetic acid, then in distilled water, siphoning off 30 minutes after each wash. The process is repeated 7-8 times. It is then dried and activated at 200° [Vogh and Thomson *AC* **53** 1365 *1981*].

Preparation of other adsorbents. Silica gel can be prepared from commercial water-glass by diluting it with water to a density of 1.19 and, while keeping it cooled to 5°, adding concentrated hydrochloric acid with stirring until the solution is acid to thymol blue. After standing for 3h, the precipitate is filtered off, washed on a Büchner funnel with distilled water, then suspended in 0.2M hydrochloric acid. The suspension is stood for 2-3days, with occasional stirring, then filtered, washed well with water and dried at 110°. It can be activated by heating up to about 200° as described for alumina.
Powdered commercial silica gel can be purified by suspending and standing overnight in concentrated hydrochloric acid (6ml/g), decanting the supernatant and repeating with fresh acid until the latter remains colourless. After filtering with suction on a sintered-glass funnel, the residue is suspended in water and washed by decantation until free of chloride ions. It is then filtered, suspended in 95% ethanol, filtered again and washed on the filter with 95% ethanol. The process is repeated with anhydrous ethyl ether before the gel is heated for 24h at 100° and stored for another 24h in a vacuum desiccator over phosphorus pentoxide.
Commercial silica gel has also been purified by suspension of 200g in 2L of 0.04M ammonia, allowed to stand for 5min before siphoning off the supernatant. The procedure was repeated 3-4 times, before rinsing with distilled water and drying and activating the silica gel in an oven at 110° [Vogh and Thomson, *AC* **53** 1345 *1981*].

Diatomaceous earth. (Celite 535 or 545, Hyflo Super-cel, Dicalite, Kieselguhr) is purified before use by washing with 3M hydrochloric acid, then water, or it is made into a slurry with hot water, filtered at the pump and washed with water at 50° until the filtrate is no longer alkaline to litmus. Organic materials can be removed by repeated extraction at 50° with methanol, benzene or chloroform, followed by washing with methanol, filtering and drying at 90-100°.

Activation of **charcoal** is generally achieved satisfactorily by heating gently to red heat in a crucible or quartz beaker in a muffle furnace, finally allowing to cool under an inert atmosphere in a desiccator. To improve the porosity, charcoal columns are usually prepared in admixture with diatomaceous earth.

Purification of **cellulose** for chromatography is by sequential washing with chloroform, ethanol, water, ethanol, chloroform and acetone. More extensive purification uses aqueous ammonia, water, hydrochloric acid, water, acetone and ethyl ether, followed by drying in a vacuum. Trace metals can be removed from filter paper by washing for several hours with 0.1M oxalic or citric acid, followed by repeated washing with distilled water.

Partition Chromatography

Partition chromatography is concerned with the distribution of substances between a mobile phase and a non-volatile liquid which is itself adsorbed onto an inert supporting stationary phase. The mobile phase may be a gas (see vapour phase chromatography) or a liquid. Paper chromatography, and reverse-phase thin layer chromatography are other applications of partition chromatography. Yet another application is paired-ion chromatography which is used for the separation of substances by virtue of their ionic properties. In principle, the separation of components of a mixture depends on the differences in their distribution ratios between the mobile phase and the liquid stationary phase. The more the distribution of a substance favours the stationary phase, the more slowly it progresses through the column.

When cellulose is used as a stationary phase, with water or aqueous organic solvents as eluents, the separation of substances is by partition between the eluting mixture and the water adsorbed on the column. This is similar to the cellulose in paper chromatography.

For chromatography on dextran gels see page 22.

Flash Chromatography

A faster method of separating components of a mixture is *flash chromatography* (see Still et al. *JOC* **43** 2923 *1978*). In flash chromatography the eluent flows through the column under a pressure of *ca* 1 to 4 atmospheres. The lower end of the chromatographic column has a relatively long taper closed with a tap. The upper end of the column is connected through a ball joint to a tap. The tapered portion is plugged with cotton, or quartz, wool and *ca* 1 cm of fine washed sand. The adsorbant is then placed in the column as a dry powder or as a slurry in a solvent and allowed to fill about one third of the column. A fine grade of adsorbant is required in order to slow the flow rate at the higher pressure, e.g. Silica 60, 230 to 400 mesh (ASTM) with particle size 0.040-0.063mm (from Merck). The top of the adsorbant is layered with *ca* 1 cm of fine washed sand. The mixture in the smallest volume of solvent is applied at the top of the column and allowed to flow into the adsorbant under gravity by opening the lower tap momentarily. The top of the column is filled with eluent, the ball joint assembled, clipped together, the upper tap is connected by a tube to a nitrogen supply from a cylinder, or to compressed air, and turned on to the desired pressure (monitor with a gauge). The lower tap is turned on and fractions are collected rapidly until the level of eluent has reached the top of the adsorbant (do not allow the column to run dry). If further elution is desired then both taps are turned off, the column is filled with more eluting solvent and the process repeated. The top of the column can be modified so that gradient elution can be performed. Alternatively, an apparatus for producing the gradient is connected to the upper tap by a long tube and placed high above the column in order to produce the required hydrostatic pressure. Flash chromatography is more efficient and gives higher resolution than conventional chromatography at atmospheric pressure and is completed in a relatively shorter time. A successful separation of components of a mixture by TLC using the same adsorbant is a good indication that flash chromatography will give the desired separation on a larger scale.

Paired-ion Chromatography

Mixtures containing ionic compounds (e.g. acids and/or bases), non-ionisable compounds, and zwitterions, can be separated successfully by paired-ion chromatography (PIC). It utilises the `reverse-phase' technique (Eksberg and Schill *AC* **45** 2092 *1973*). The stationary phase is lipophilic, such as μ-BONDAPAK C_{18} (Waters Assoc) or any other adsorbent that is compatible with water. The mobile phase is water or aqueous methanol containing the acidic or basic counter ion. Thus the mobile phase consists of dilute solutions of strong acids (e.g. 5mM 1-heptanesulphonic acid) or strong bases (e.g. 5 mM tetrabutylammonium phosphate) that are completely ionised at the operating *p*H values which are usually between 2 and 8. An equilibrium is set up between the neutral species of a mixture in the stationary phase and the respective ionised (anion or cation) species which dissolve in the mobile phase containing the counter ions. The extent of the equilibrium will depend on the ionisation constants of the respective components of the mixture, and the solubility of the unionised species in the stationary phase. Since the ionisation constants and the solubility in the stationary phase will vary with the water-methanol ratio of the mobile phase, the separation may be improved by altering this ratio gradually (gradient elution) or stepwise. If the compounds are eluted too rapidly the water content of the mobile phase should be increased, e.g. by steps of 10%. Conversely, if components do not move, or move slowly, the methanol content of the mobile phase should be increased by steps of 10%.

The application of pressure to the liquid phase in liquid chromatography generally increases the separation (see HPLC). Also in PIC improved efficiency of the column is observed if pressure is applied to the mobile phase (Wittmer, Nuessle and Haney *AC* **47** 1422 *1975*).

Ion-exchange Chromatography

Ion-exchange chromatography involves an electrostatic process which depends on the relative affinities of various types of ions for an immobilised assembly of ions of opposite charge. The stationary phase is an aqueous buffer with a fixed pH or an aqueous mixture of buffers in which the pH is continuously increased or decreased as the separation may require. This form of liquid chromatography can also be performed at high inlet pressures of liquid with increased column performances.

Ion-exchange Resins. An ion-exchange resin is made up of particles of an insoluble elastic hydrocarbon network to which is attached a large number of ionisable groups. Materials commonly used comprise synthetic ion-exchange resins made, for example, by crosslinking polystyrene to which has been attached non-diffusible ionised or ionisable groups. Resins with relatively high crosslinkage (8-12%) are suitable for the chromatography of small ions, whereas those with low crosslinkage (2-4%) are suitable for larger molecules. Applications to hydrophobic systems are possible using aqueous gels with phenyls bound to the rigid matrix (Phenyl-Superose, Pharmacia) or neopentyl chains (Alkyl-Superose, Pharmacia). (Superose is a cross-linked agarose-based medium with an almost uniform bead size.) These groups are further distinguishable as strong ($-SO_2OH$, $-NR_3^+$) or weak ($-OH$, $-CO_2H$, $-PO(OH)_2$, $-NH_2$). Their charges are counterbalanced by diffusible ions, and the operation of a column depends on its ability and selectivity to replace these ions. The exchange that takes place is primarily an electrostatic process but adsorptive forces and hydrogen bonding can also be important. A typical sequence for the relative affinities of some common anions (and hence the inverse order in which they pass through such a column), is the following, obtained using a quaternary ammonium (strong base) anion-exchange column:

Fluoride < acetate < bicarbonate < hydroxide < formate < chloride < bromate < nitrite < cyanide < bromide < chromate < nitrate < iodide < thiocyanate < oxalate < sulphate < citrate.

For an amine (weak base) anion-exchange column in its chloride form, the following order has been observed:

Fluoride < chloride < bromide = iodide = acetate < molybdate < phosphate < arsenate < nitrate < tartrate < citrate < chromate < sulphate < hydroxide.

With strong cation-exchangers, the usual sequence is that polyvalent ions bind more firmly than mono- or di- valent ones, a typical series being as follows:

$Th^{4+} > Fe^{3+} > Al^{3+} > Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Co^{2+} > Ni^{2+} = Cu^{2+} > Zn^{2+} = Mg^{2+} > UO_2^{+} = Mn^{2+} > Ag^{+} > Tl^{+} > Cs^{+} > Rb^{+} > NH_4^{+} = K^{+} > Na^{+} > H^{+} > Li^{+}$.

Thus, if an aqueous solution of a sodium salt contaminated with heavy metals is passed through the sodium form of such a column, the heavy metal ions will be removed from the solution and will be replaced by sodium ions from the column. This effect is greatest in dilute solution. Passage of sufficiently strong solutions of alkali metal salts or mineral acids readily displaces all other cations from ion-exchange columns. (The regeneration of columns depends on this property.) However, when the cations lie well to the left in the above series it is often advantageous to use a complex-forming species to facilitate removal. For example, iron can be displaced from ion-exchange columns by passage of sodium citrate or sodium ethylenediaminetetraacetate.

Some of the more common commercially available resins are listed in Table 13.

Ion-exchange resins swell in water to an extent which depends on the amount of crosslinking in the polymer, so that columns should be prepared from the wet material by adding it as a suspension in water to a tube already partially filled with water. (This also avoids trapping air bubbles,) The exchange capacity of a resin is commonly expressed as mg equiv./ml of wet resin. This quantity is pH-dependent for weak-acid or weak-base resins but is constant at about 0.6-2 for most strong-acid or strong-base types.

Apart from their obvious applications to inorganic species, sulphonic acid resins have been used in purifying amino acids, aminosugars, organic acids, peptides, purines, pyrimidines, nucleosides, nucleotides and polynucleotides. Thus, organic bases can be applied to the H^+ form of such resins by adsorbing them from neutral solution and, after washing with water, they are eluted sequentially with suitable buffer solutions or dilute acids. Alternatively, by passing alkali solution through the column, the bases will be displaced in an order that is governed by their pK values. Similarly, strong-base anion exchangers have been used for aldehydes and ketones (as bisulphite addition compounds), carbohydrates (as their borate complexes), nucleosides, nucleotides, organic acids, phosphate esters and uronic acids. Weakly acidic and weakly basic exchange resins have also found extensive applications, mainly in resolving weakly basic and acidic species. For demineralisation of solutions without large changes in pH, mixed-bed resins can be prepared by mixing a cation-exchange resin in its H^+ form with an anion-exchange resin in its OH^- form. Commercial examples include Amberlite MB-1 (IR-120 + IRA-400) and Bio-Deminrolit (Zeo-Karb 225 and Zerolit FF). The latter is also available in a self-indicating form.

Ion-exchange Celluloses and Sephadex. A different type of ion-exchange column that is finding extensive application in biochemistry for the purification of proteins, nucleic acids and acidic polysaccharides derives from cellulose by incorporating acidic and basic groups to give ion-exchangers of controlled acid and basic strengths. Commercially available cellulose-type resins are given in Tables 14 and 15. AG 501 x 8 (Bio-Rad) is a mixed-bed resin containing equivalents of AG 50W-x8 H^+ form and AG 1-x8 OH^- form, and Bio-Rex MSZ 501 resin. A dye marker indicates when the resin is exhausted. Removal of unwanted cations, particularly of the transition metals, from amino acids and buffer can be achieved by passage of the solution through a column of Chelex 20 or Chelex 100. The metal-

acids and buffer can be achieved by passage of the solution through a column of Chelex 20 or Chelex 100. The metal-chelating abilities of the resin reside in the bonded iminodiacetate groups. Chelex can be regenerated by washing in two bed volumes of 1M HCl, two bed volumes of 1M NaOH and five bed volumes of water.
Ion-exchange celluloses are available in different particle sizes. It is important that the amounts of 'fines' are kept to a minimum otherwise the flow of liquid through the column can be extremely slow and almost stop. Celluloses with a large range of particle sizes should be freed from 'fines' before use. This is done by suspending the powder in the required buffer and allowing it to settle for one hour and then decanting the 'fines'. This separation appears to be wasteful but it is necessary for reasonable flow rates without applying high pressures at the top of the column. Good flow rates can be obtained if the cellulose column is packed dry whereby the 'fines' are evenly distributed throughout the column. Wet packing causes the fines to rise to the top of the column, which thus becomes clogged.
Several ion-exchange celluloses require recycling before use, a process which must be applied for recovered celluloses. Recycling is done by stirring the cellulose with 0.1M aqueous sodium hydroxide, washing with water until neutral, then suspending in 0.1M hydrochloric acid and finally washing with water until neutral. When regenerating a column it is advisable to wash with a salt solution (containing the required counter ions) of increasing ionic strength up to 2M. The cellulose is then washed with water and recycled if necessary. Recycling can be carried out more than once if there are doubts about the purity of the cellulose and when the cellulose had been used previously for a different purification procedure than the one to be used. The basic matrix of these ion-exchangers is cellulose and it is important not to subject them to strong acid (> 1M) and strongly basic (> 1M) solutions.
When storing ion-exchange celluloses, or during prolonged usage, it is important to avoid growth of microorganisms or moulds which slowly destroy the cellulose. Good inhibitors of microorganisms are phenyl mercuric salts (0.001%, effective in weakly alkaline solutions), chlorohexidine (Hibitane at 0.002% for anion exchangers), 0.02% aqueous sodium azide or 0.005% of ethyl mercuric thiosalicylate (Merthiolate) are most effective in weakly acidic solutions for cation exchangers. Trichlorobutanol (Chloretone, at 0.05% is only effective in weakly acidic solutions) can be used for both anion and cation exchangers. Most organic solvents (e.g. methanol) are effective antimicrobial agents but only at high concentrations. These inhibitors must be removed by washing the columns thoroughly before use because they may have adverse effects on the material to be purified (e.g. inactivation of enzymes or other active preparations).

In recent years other carbohydrate matrices such as *Sephadex* (based on dextran) have been developed which have more uniform particle sizes. Their advantages over the celluloses include faster and more reproducible flow rates and they can be used directly without removal of 'fines'.
Sephadex, which can also be obtained in a variety of ion-exchange forms (see Table 15) consists of beads of a cross-linked dextran gel which swells in water and aqueous salt solutions. The smaller the bead size the higher the resolution that is possible but the slower the flow rate. Typical applications of Sephadex gels are the fractionation of mixtures of polypeptides, proteins, nucleic acids, polysaccharides and for desalting solutions.
Sephadex is a bead form of cross-linked dextran gel. *Sepharose CL* and *Bio-Gel A* are derived from agarose. Sephadex ion-exchangers, unlike celluloses, are available in narrow ranges of particle sizes. These are of two medium types, the G-25 and G-50, and their dry bead diameter sizes are *ca* 50 to 150 microns. They are available as cation and anion exchange Sephadex. One of the disadvantages of using Sephadex ion-exchangers is that the bed volume can change considerably with alteration of pH. *Ultragels* also suffer from this disadvantage to a varying extent, but ion-exchangers of the bead type have been developed e.g. *Fractogels, Toyopearl*, which do not suffer from this disadvantage.

Sepharose (e.g. *Sepharose CL and Bio-Gel A*) is a bead form of agarose gel which is useful for the fractionation of high molecular weight substances, for molecular weight determinations of large molecules (molecular weight > 5000), and for the immobilisation of enzymes, antibodies, hormones and receptors usually for affinity chromatography applications.
In preparing any of the above for use in columns, the dry powder is evacuated, then mixed under reduced pressure with water or the appropriate buffer solution. Alternatively it is stirred gently with the solution until all air bubbles are removed. Because some of the wet powders change volumes reversibly with alteration of pH or ionic strength (see above), it is imperative to make allowances when packing columns (see above) in order to avoid overflowing of packing when the pH or salt concentrations are altered.

Cellex CM ion-exchange cellulose can be purified by treatment of 30-40g (dry weight) with 500ml of 1mM cysteine hydrochloride. It is then filtered through a Büchner funnel and the filter cake is suspended in 500ml of 0.05M NaCl/0.5M NaOH. This is filtered and the filter cake is resuspended in 500ml of distd water and filtered again. The process is repeated until the washings are free from chloride ions. The filter cake is again suspended in 500ml of 0.01M buffer at the desired pH for chromatography, filtered, and the last step repeated several times.
Cellex D and other anionic celluloses are washed with 0.25M NaCl/0.25M NaOH solution, then twice with deionised water. This is followed with 0.25M NaCl and then washed with water until chloride-free. The Cellex is then equilibrated with the desired buffer as above.

Crystalline Hydroxylapatite is a structurally organised, highly polar material which, in aqueous solution (in buffers) strongly adsorbs macromolecules such as proteins and nucleic acids, permitting their separation by virtue of the interaction with charged phosphate groups and calcium ions, as well by physical adsorption. The

procedure therefore is not entirely ion-exchange in nature. Chromatographic separations of singly and doubly stranded DNA are readily achievable whereas there is negligible adsorption of low molecular weight species.

Gel Filtration

The gel-like, bead nature of wet Sephadex (a modified dextran) enables small molecules such as inorganic salts to diffuse freely into it while, at the same time, protein molecules are unable to do so. Hence, passage through a Sephadex column can be used for complete removal of salts from protein solutions. Polysaccharides can be freed from monosaccharides and other small molecules because of their differential retardation. Similarly, amino acids can be separated from proteins and large peptides.

Gel filtration using Sephadex G-types (50 to 200, from Pharmacia, Uppsala, Sweden) is essentially useful for fractionation of large molecules with molecular weights above 1000. For Superose (Pharmacia) the range is given as 5000 to 5 x 10^6. Fractionation of lower molecular weight solutes (e,g, ethylene glycols, benzyl alcohols) can now be achieved with Sephadex G-10 (up to Mol.Wt 700) and G-25 (up to Mol.Wt 1500). These dextrans are used only in aqueous solutions. More recently, however, Sephadex LH-20 and LH-60 (prepared by hydroxypropylation of Sephadex) have become available and are used for the separation of small molecules (Mol.Wt less than 500) using most of the common organic solvents as well as water.

Sephasorb HP (ultrafine, prepared by hydroxypropylation of crossed-linked dextran) can also be used for the separation of small molecules in organic solvents and water, and in addition it can withstand pressures up to 1400 psi making it useful in HPLC. Because solutions with high and low pH values slowly decompose, these gels are best operated at pH values between 2 and 12 (see further in Chapter 5).

High Performance Liquid Chromatography (HPLC)

When pressure is applied at the inlet of a liquid chromatographic column the performance of the column can be increased by several orders of magnitude. This is partly because of the increased speed at which the liquid flows through the column and partly because fine column packings can be used which have larger surface areas. Because of the improved efficiency of the columns this technique has been referred to as high performance, high pressure, or high speed liquid chromatography.

Equipment consists of a hydraulic system to provide the pressure at the inlet of the column, a column, a detector and a recorder. The pressures used in HPLC vary from a few psi to 4000-5000 psi. The most convenient pressures are, however, between 500 and 1800psi. The plumbing is made of stainless steel or non-corrosive metal tubing to withstand high pressures. Plastic tubing and connectors are used for low pressures, e.g. up to ~500psi. Increase of temperature has a very small effect on the performance of a column in liquid chromatography. Small variations in temperatures, however, do upset the equilibrium of the column, hence it is advisable to place the column in an oven at ambient temperature in order to achieve reproducibility. The packing (stationary phase) is specially prepared for withstanding high pressures. It may be an adsorbent (for adsorption or solid-liquid HPLC), a material impregnated with a high boiling liquid (e.g. octadecyl sulphate, in *reverse-phase* or *liquid-liquid or paired-ion* HPLC), an ion-exchange material (in *ion-exchange* HPLC), or a highly porous non-ionic gel (for high performance *gel filtration*). The mobile phase is water, aqueous buffers, salt solutions, organic solvents or mixtures of these. The more commonly used detectors have UV, visible, or fluorescence monitoring for light absorbing substances, and refractive index monitoring for transparent compounds. The sensitivity of the refractive index monitoring is usually lower than the light absorpting monitoring by a factor of ten or more. The cells of the monitoring devices are very small (*ca* 5 µl) and the detection is very good. The volumes of the analytical columns are quite small (*ca* 2ml for a 1 metre column) hence the result of an analysis is achieved very quickly. Larger columns have been used for preparative work and can be used with the same equipment. Most modern machines have solvent mixing chambers for solvent gradient or ion gradient elution. The solvent gradient (for two solvents) or pH or ion gradient can be adjusted in a linear, increasing or decreasing exponential manner. Some of the more common column packings are listed in Table 16.

Purification of stereoisomers has been achieved by applying HPLC using a chiral stationary phase such as (*R*)-*N*-3,5-dinitrobenzoylphenylglycine or (*S*)-3,5-dinitrobenzoylleucine. Examples covering a wide range of compounds are given in references by Pirkle et al. in *JACS* **103** 3964 *1981*, and *ACS Symposium Series no 185, "Asymmetric Reactions and Processes in Chemistry"*, Eliel and Otsuka eds (*Amer Chem Soc*, Washington DC, *pp* 245-260, *1982*); see more recent references on *chiral chromatography* on p 44.

Other Types of Liquid Chromatography

New stationary phases for specific purposes in chromatographic separation are being continually proposed. *Charge transfer adsorption chromatography* makes use of a stationary phase which contains immobilised aromatic compounds and permits the separation of aromatic compounds by virtue of the ability to form charge

transfer complexes (sometimes coloured) with the stationary phase. The separation is caused by the differences in stability of these complexes (Porath and Dahlgren-Caldwell *JC* **133** 180 *1977*).
In *metal chelate adsorption chromatography* a metal is immobilised by partial chelation on a column which contains bi- or tri- dentate ligands. Its application is in the separation of substances which can complex with the bound metals and depends on the stability constants of the various ligands (Porath, Carlsson, Olsson and Belfrage *Nature* **258** 598 *1975*; Loennerdal, Carlsson and Porath *FEBS LETT* **75** 89 *1977*).
An application of chromatography which has found extensive use in biochemistry and has brought a new dimension in the purification of enzymes is *affinity chromatography*. A specific enzyme inhibitor is attached by covalent bonding to a stationary phase (e.g. AH-Sepharose 4B for acidic inhibitors and CH-Sepharose 4B for basic inhibitors), and will strongly adsorb only the specific enzyme which is inhibited, allowing all other proteins to flow through the column. The enzyme is then eluted with a solution of high ionic strength (e.g. 1M sodium chloride) or a solution containing a substrate or reversible inhibitor of the specific enzyme. (The ionic medium can be removed by gel filtration using a mixed-bed gel.) Similarly, an immobilised lectin may interact with the carbohydrate moiety of a glycoprotein. The most frequently used matrixes are cross-linked (4-6%) agarose and polyacrylamide gel. Many adsorbents are commercially available for nucleotides, coenzymes and vitamins, amino acids, peptides and lectins. Considerable purification can be achieved by one passage through the column and the column can be reused several times.
The affinity method may be *biospecific*, for example as an antibody-antigen interaction chemical as in the chelation of boronate by *cis*-diols, or of unknown origin as in the binding of certain dyes to albumin.

Hydrophobic adsorption chromatography takes advantage of the hydrophobic properties of substances to be separated and has also found use in biochemistry (Hoftsee *BBRC* **50** 751 *1973*; Jennissen and Heilmayer Jr *Biochemistry* **14** 754 *1975*). Specific covalent binding with the stationary phase, a procedure that was called *covalent chromatography*, has been used for separation of compounds and for immobilising enzymes on a support: the column was then used to carry out specific bioorganic reactions (Mosbach *Methods in Enzymology* **44**, 1976; A.Rosevear, J.F.Kennedy and J.M.S.Cabral, *Immobilised Enzymes and Cells: A Laboratory Manual*, Adam Hilger, Bristol, 1987).

Vapour Phase Chromatography

The mobile phase in vapour phase chromatography is a gas (e.g. hydrogen, helium, nitrogen or argon) and the stationary phase is a non-volatile liquid impregnated onto a porous material. The mixture to be purified is injected into a heated inlet whereby it is vaporised and taken into the column by the carrier gas. It is separated into its components by partition between the liquid on the porous support and the gas. For this reason vapour-phase chromatography is sometimes referred to as gas-liquid chromatography.

Although this technique was first used for analytical purposes in 1952, its application to the purification of chemicals at a preparative level is much more recent and commercial instruments for this purpose are currently in a state of rapid development. This type of partition chromatography uses a tubular column packed with an inert material which is impregnated with a liquid. This liquid separates components of gases or vapours as they flow through the column. On a preparative scale, use of a large column heated slightly above the boiling point of the material to be processed makes it possible to purify in this way small quantities of many volatile organic substances. For example, if the impurities have a greater affinity for the liquid in the column than the desired component has, the latter will emerge first and in a substantially pure form.
In operation, the organic material is carried as a vapour in a *carrier* gas such as hydrogen, helium, carbon dioxide, nitrogen or argon (in a manner analogous to a solution in a suitable solvent in liquid chromatography). The technique that is almost invariably used is to inject the substance (for example, by means of a hypodermic syringe) over a relatively short time on to the surface of the column through which is maintained a slow continuous passage of the chemically inert carrier gas. This leads to the progressive elution of individual components from the column in a manner analogous to the movement of bands in conventional chromatography. As substances emerge from the column they can be condensed in suitable traps. The carrier gas blows the vapour through these traps hence these traps have to be very efficient. Improved collection of the effluent vaporised fractions in preparative work is attained by strong cooling, increasing the surface of the traps by packing them with glass wool, and by applying an electrical potential which neutralises the charged vapour and causes it to condense.
The choice of carrier gas is largely determined by the type of detection system that is available (see below). Column efficiency is greater in argon, nitrogen or carbon dioxide than it is in helium or hydrogen, but the latter are less impeded by flowing through packed columns so that lower pressure differentials exist between inlet and outlet. The packing in the column is usually an inert supporting material such as powdered firebrick, or a firebrick-Celite mixture

coated with a high-boiling organic liquid as the stationary phase. These liquids include Apiezon oils and greases, di-esters (such as dibutylphthalate or di-2-ethylhexyl sebacate), polyesters (such as diethyleneglycol sebacate), polyethylene glycols, hydrocarbons (such as Nujol or squalene), silicone oils and tricresyl phosphate.
The coating material (about 75ml per 100ml of column packing) is applied as a solution in a suitable solvent such as methylene chloride, acetone, methanol or pentane, which is then allowed to evaporate in air, over a steam-bath, or in a vacuum oven (provided the adsorbed substance is sufficiently non-volatile). The order in which a mixture of substances travels through such columns depends on their relative solubilities in the materials making up the stationary phases.

Stationary Phase	Mixture
Benzyl diphenyl	Aromatic molecules
Benzyl ether	Saturated hydrocarbons and olefines
Bis(2-*n*-butoxyethyl)phthalate	Saturated hydrocarbons and olefines
Diethylene glycol adipate	Methyl esters of fatty acids up to C_{24}
Dimethyl sulpholane (below 40°)	Saturated and unsaturated hydrocarbons
Dinonyl phthalate	Paraffins, olefines, low molecular weight aromatics, alcohols (up to amyl alcohol), lower ethers, esters and carbonyl compounds.
Hexadecane	Low-boiling hydrocarbons
Mineral oil	Aliphatic and aromatic amines
2,2'-Oxydipropionitrile	Paraffins, cycloalkanes, olefines, ethers, alkylbenzenes, acetates, aldehydes, alcohols, acetals and ketones
Polyethylene glycols	Aromatic molecules from paraffins
Silicone oil	Aromatic hydrocarbons, alcohols, esters
Silicone-stearic acid	Fatty acids
Squalane	Saturated hydrocarbons
Tricresyl phosphate	Hexanes, heptanes, aromatics, organic sulphur compounds and aliphatic chlorides

The three main requirements of a liquid for use in a gas chromatograph column are that it must have a high boiling point, a low vapour pressure, and at the same time permit adequate separation of components fairly rapidly. As a rough guide, the boiling point should be at least 250° above the temperature of the column, and, at column temperatures, the liquid should not be too viscous, nor should it react chemically with the sample. Liquids suitable for use as stationary phases in gas chromatography are given in Table 17 and above.
Where the stationary phase is chemically similar to the material to be separated, the main factors governing the separation will be the molecular weight and the shape. Otherwise, polar interactions must also be considered, for example hydroxylated compounds used for stationary phases are likely to retard the movement through the column of substances with hydrogen accepting groups. A useful guide to the selection of a suitable stationary phase is to compare, on the basis of polarity, possible materials with the components to be separated. This means that, in general, solute and solvent will be members of the same, or of adjacent, classes in the following groupings:

A. Water, polyhydric alcohols, aminoalcohols, oxyacids, polyphenols, di- and tri-carboxylic acids.
B. Alcohols, fatty acids, phenols, primary and secondary amines, oximes, nitro compounds, nitriles with α-H atoms.
C. Ethers, ketones, aldehydes, esters, tertiary amines, nitriles without α-H atoms.
D. Chlorinated aromatic or olefinic hydrocarbons.
E. Saturated hydrocarbons, carbon disulphide, tetrachloromethane.

Material emerging from the column is detected by a thermal-conductivity cell, an ionisation method, or a gas-density balance.
The first of these methods, which is applicable when hydrogen or helium is used as carrier gas, depends on the differences in heat conductivities between these gases and most others, including organic substances. The resistance of a tungsten or platinum wire heated by a constant electric current will vary with its temperature which, in turn, is a function of the thermal conductivity through the gas. These devices, also known as catharometers, can detect about 10^{-8} moles of substance. When argon is used as carrier gas, an ionisation method is practicable. It is based on measurement of the current between two electrodes at different voltages in the presence of a suitable emitter of ß-radiation. The gas-density balance method depends on measurement of the difference in thermal e.m.f. between two equally warmed copper-constantan thermocouples located in the cross-channel of what constitutes a mechanical equivalent to the Wheatstone bridge. Any increase in density of the effluent gas relative to the reference gas will cause

movement of gas along the cross-channel, and hence cool one of the thermocouples relative to the other. The technique is comparable in sensitivity with the thermal-conductivity method.

More recently *glass capillary columns* have been used. These columns can be several metres long. The glass capillary wall acts as the support onto which is coated the liquid phase. These columns have much superior separating powers than the conventional columns. In some cases the resolution is so good that enantiomeric and diastereomeric compounds have been separated. When these columns are attached to a mass spectrometer a very powerful analytical tool (*gas chromatography-mass spectrometry*; **GC-MS**) is produced. Because of the relatively small amounts of material required for mass spectrometry, a splitting system is inserted between the column and the mass spectrometer. This enables only a small fraction of the effluent to enter the spectrometer, the rest of the effluent is usually vented to the air. Even more recently a liquid chromatographic column has replaced the gas chromatographic column in the chromatography-mass spectrometry analyses

Paper Chromatography

Paper chromatography is basically a type of partition chromatography between water adsorbed onto the cellulose fibre of the paper and a liquid mobile phase in a closed tank. The most common application is the ascending solvent technique. The paper is hung by means of clips or string and the lower end is made to dip into the eluting solvent. The material under test is applied as a spot 2.5 cm or so above the lower end of the paper and marked with a pencil. It is important that the spots are above the eluting solvent before it begins to rise up the paper by capillarity. Eluents are normally aqueous mixtures of organic solvents, acids or bases. (For solvent systems see Lederer and Lederer, p 44). The descending technique has also been used, and in this case the top of the paper dips into a trough containing the eluent which travels downwards, also by capillarity. The spots are applied at the top of the paper close to the solvent trough. A closed tank is necessary for these operations because better reproducibility is achieved if the solvent and vapour in the tank are in equilibrium. The tanks have to be kept away from draughts. Elution times vary from several hours to a day depending on the solvent system and paper. For more efficient separations the dried paper is eluted with a different solvent along a direction which is 90° from that of the first elution. This is referred as *two dimensional paper chromatography*. In a third application (*circular paper chromatography*) ordinary circular filter papers are used. The filter paper is placed between two glass plates. The upper plate has a hole in the centre which is coincident with the centre of the paper. A strong solution of the mixture is then separated radially by the eluting solvent. A strong solution of the mixture is placed in this hole followed by the eluting solvent. After the solvents have travelled the required distances in the above separations, the papers are air dried and the spots are revealed by their natural colours or, by spraying with a reagent that forms a coloured product with the spots. In many cases, the positions of the spots can be seen as light fluorescing or absorbing spots when viewed under UV light.
The use of *thick paper* such as Whatman nos 3 or 31 (0.3-0.5mm) increases the amounts that can be handled (up to about 100mg per sheet). Larger quantities require multiple sheets or cardboard, e.g. Scheicher and Schüll nos 2071 (0.65mm), 2230 (0.9mm) or 2181 (4mm). For even larger amounts recourse may be had to *chromatopack* or *chromatopile* procedures. The latter use a large number (200-500) of identical filter papers stacked and compressed in a column, the material to be purified being adsorbed onto a small number of these discs which, after drying, are placed almost at the top of the column. The column is then subjected to descendiing development, and bands are separated mechanically by disassembling the filter papers. Instead of filter papers, *cellulose powder* may be suitable, the column being packed by first suspending the powder in the solvent to be used for development. Yet another variation employs tightly wound *paper roll columns* contained in thin polythene skins. (These are unsuitable for such solvents as benzene, chloroform, collidine, ethyl ether, pyridine and toluene).
The technique of paper chromatography has been almost entirely superseded by thin- or thick-layer chromatography (see below).

Thin or Thick Layer Chromatography (TLC)

Thin layer chromatography is in principle similar to paper chromatography when used in the ascending method, i.e. the solvent creeps up the stationary phase by capillarity. The adsorbent (e.g. silica, alumina, cellulose) is spread on a rectangular glass plate (or solid inert plastic sheet). Some adsorbents (e.g. silica) are mixed with a setting material (e.g. $CaSO_4$) by the manufacturers which causes the film to set on drying. The adsorbent can

be activated by heating at 100-110° for a few hours. Other adsorbents (e.g. celluloses) adhere on glass plates without a setting agent. The materials to be purified are spotted in the solvent close to the lower end of the plate and allowed to dry. The spots will need to be placed at such a distance as to ensure that when the lower end of the plate is immersed in the solvent, the spots are a few mm above the eluting solvent. The plate is placed upright in a tank containing the eluting solvent. Elution is carried out in a closed tank as in paper chromatography to ensure equilibrium. It requires less than three hours for the solvent to reach the top of the plate. Good separations can be achieved with square plates if a second elution is performed at right angles to the first as in two dimensional paper chromatography. For rapid work plates of the size of microscopic slides or even smaller are used which can decrease the elution time to as little as fifteen minutes without loss of resolution. The advantage of plastic backed plates is that the size of the plate can be made as required by cutting the sheet with scissors.

The thickness of the plates could be between 0.2mm to 2mm or more. The thicker plates are used for preparative work in which hundreds of milligrams of mixtures can be purified conveniently and quickly. The spots or areas are easily scraped off the plates and eluted with the required solvent. These can be revealed on the plates by UV light if they are UV absorbing or fluorescing substances, by spraying with a reagent that gives coloured products with the spot (e.g. iodine solution or vapour gives brown colours with amines), or with dilute sulphuric acid (organic compounds become coloured or black when the plates are heated at 100°) if the plates are of alumina or silica, but not cellulose. Some alumina and silica powders are available with fluorescent materials in them, in which case the whole plate fluoresces under UV light. Non-fluorescing spots are thus clearly visible, and fluorescent spots invariably fluoresce with a different colour. The colour of the spots can be different under UV light at 254nm and at 365nm. Another useful way of showing up non-UV absorbing spots is to spray the plate with a 1-2% solution of Rhodamine 6G in acetone. Under UV light the dye fluoresces and reveals the non-fluorescing spots. If the material in the spot is soluble in ether, benzene or light petroleum, the spots can be extracted from the powder with these solvents which leave the water soluble dye behind.

Thin and thick layer chromatography have been used successfully with ion-exchange celluloses as stationary phases and various aqueous buffers as mobile phases. Also, gels (e.g. Sephadex G-50 to G-200 superfine) have been adsorbed on glass plates and are good for fractionating substances of high molecular weights (1500 to 250,000). With this technique, which is called *thin layer gel filtration* (**TLG**), molecular weights of proteins can be determined when suitable markers of known molecular weights are run alongside.

Commercially available precoated plates with a variety of adsorbents are generally very good for quantitative work because they are of a standard quality. More recently plates of a standardised silica gel 60 (as medium porosity silica gel with a mean porosity of 6mm) were released by Merck. These have a specific surface of 500 m^2/g and a specific pore volume of 0.75 ml/g. They are so efficient that they have been called *high performance thin layer chromatography* (**HPTLC**) plates (Ropphahn and Halpap *JC* **112** 81 *1975*). In another variant of thin layer chromatography the adsorbent is coated with an oil as in gas chromatography thus producing *reverse-phase thin layer chromatography.*

A very efficient thin layer form of circular paper chromatography makes use of a circular glass disc coated with an adsorbent (silica, alumina or cellulose). The apparatus is called a **Chromatotron** (available from Harrison Research, USA). The disc is rotated by a motor, and the sample followed by the eluting solvent are allowed to drip onto a central position on the plate. As the plate rotates the solvent elutes the mixture, centrifugally, while separating the components in the form of circles radiating from the central point. When elution is complete the revolving circular plate is stopped and the circular bands are scraped off and extracted with a suitable solvent.

SOLVENT EXTRACTION AND DISTRIBUTION

Extraction of a substance from suspension or solution into another solvent can sometimes be used as a purification process. Thus, organic substances can often be separated from inorganic impurities by shaking an aqueous solution or suspension with suitable immiscible solvents such as benzene, carbon tetrachloride, chloroform, ethyl ether, isopropyl ether or petroleum ether. After several such extractions the combined organic phase is dried and the solvent is evaporated. Grease from the glass taps of conventional separating funnels is invariably soluble in the solvents used. Contamination with grease can be very troublesome particularly when the amounts of material to be extracted are very small. Instead, the glass taps should be lubricated with the extraction solvent; or better, the taps of the extraction funnels should be made of the more expensive material *Teflon*. Immiscible solvents suitable for extractions are given in Table 18. Addition of electrolytes (such as ammonium sulphate, calcium chloride or sodium chloride) to the aqueous phase helps to ensure that the organic

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